EMBRYOLOGY OF TRIBE GYNOTROCHEAE (RHIZOPHORACEAE) AND ITS DEVELOPMENTAL AND SYSTEMATIC IMPLICATIONS[1]

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ABSTRACT

*A complete embryological description of the four genera traditionally circumscribed as tribe Gynotrocheae (*Carallia, Crossostylis, Gynotroches, *and* Pellacalyx*) is presented. Most of the character states are consistent with those known for other genera of the family that have been studied, but several are not. In* Crossostylis, Gynotroches, *and* Pellacalyx, *microsporogenesis occurs by both simultaneous and successive meiotic cytokinesis, even in a single flower. This is apparently previously unreported in any angiosperm and suggests that meiosis in the anther may be under tapetal, not sporocytic, control. Dioecy in* Gynotroches *results from late-developmental phenomena: in female flowers sporopollenin pollen walls fail to form, and in male flowers late ovule development and megagametogenesis are abnormal. Embryological and other morphological data indicate that Gynotrocheae are paraphyletic and could be narrowed to only* Gynotroches *and* Pellacalyx, *which are distinct from all other Rhizophoraceae in having tenuinucellate ovules and outer integuments that remain biseriate throughout ovule and seed development.* Crossostylis *is intermediate between these two genera and the ancestral tribe Macarisieae, and* Carallia *is probably intermediate between all other inland genera and the mangrove tribe (Rhizophoreae).*

The family Rhizophoraceae is generally accepted as comprising three tribes: Macarisieae (six or seven inland genera), Gynotrocheae (four inland genera), and Rhizophoreae (four mangrove genera). The four genera previously included as tribe Anisophylleeae or as a subfamily are now segregated as an unrelated family, a disposition originally suggested by Ridley (1922) and now supported by many lines of evidence (Behnke, 1982; Tobe & Raven, 1987a; Juncosa & Tomlinson, 1987, this volume).

Embryological evidence (Tobe & Raven, 1987a) and some aspects of vegetative anatomy (Juncosa & Tomlinson, this volume) suggest that the Anisophylleaceae may be related to Myrtales, but the infrafamilial systematics and extrafamilial phylogenetic relationships of the Rhizophoraceae remain uncertain. Much of this uncertainty results from the great variability in many systematic characters in this family and the paucity of information about certain key genera. While many later-developmental and sporophytic characters exhibit considerable adaptive radiation, embryological characters are usually more conservative. Furthermore, because embryological characters are inherently developmental, homologies are more reliable and the polarity of the characters can often be determined, lending additional weight to a phylogenetic hypothesis.

The Gynotrocheae are of pivotal systematic importance within the context of the family for several reasons. There is greater variability in conventional taxonomic characters within this tribe than in the other two: phyllotaxy, wood, flowers, fruits, and seeds all afford good examples (Schimper, 1893; van Vliet, 1976; Juncosa & Tomlinson, this volume). It has even been suggested that *Pellacalyx* could be excluded from the family (Marco, 1935; Dahlgren, pers. comm.). In another direction, the floral morphology of the Rhizophoreae is very sim-

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TABLE 1. *Collection localities and voucher information for species studied.*

Species	Locality	Collection Number	Location of Voucher
Carallia borneensis Oliver	Andulau Forest Reserve, Brunei	*Juncosa 464*	(lost)
Crossostylis grandiflora Brongn. & Gris.	Mt. Panie, New Caledonia	*Juncosa 388*	NOUM, DUKE
	Yate, New Caledonia	*Juncosa 413*	NOUM, DUKE
	New Caledonia	*G. McPherson 1617*	MO
Gynotroches axillaris Blume[1]	Padawan, Sarawak, Malaysia	*Juncosa 481, 482*	SAR, DUKE
	Kuching vicinity, Sarawak, Malaysia	*Juncosa 440, 442*	SAR, DUKE
	Maxwell Hill, Perak, Malaysia	*B. C. Stone 15397*	KLU
Pellacalyx cristatus Hemsl.	Kampong Tepoi vicinity, Sarawak	*Juncosa 488*	SAR, DUKE
P. lobbii (Hook. f.) Schimp.	Andulau Forest Reserve, Brunei	*Juncosa 465*	(lost)
P. cf. *saccardianus* Scort.	Maxwell Hill, Perak, Malaysia	*B. C. Stone 15396*	KLU
P. symphiodiscus Stapf	Kuching vicinity, Sarawak, Malaysia	*Juncosa 486*	SAR, DUKE

[1] Material from the two Sarawak localities represented two distinct species (see text for details).

ilar to that of *Carallia* (Juncosa & Tomlinson, 1987; Juncosa, pers. obs.); so clues to the evolution of vivipary might be found indirectly among the embryological characteristics of this and other genera of the Gynotrocheae. Although data are not yet available for all genera of Macarisieae, they comprise a relatively homogeneous tribe in other respects (Schimper, 1893; Hutchinson & Dalziel, 1954; Floret, 1976; van Vliet, 1976; Juncosa & Tomlinson, this volume), and comprehensive embryological data for *Cassipourea* and *Sterigmapetalum* are available (Juncosa, 1984a; Tobe & Raven, 1987b). Fragmentary data are available for two genera of Gynotrocheae (Mauritzon, 1939), and seed coat anatomy of all four genera is now well known (Corner, 1976; Tobe & Raven, this volume).

MATERIALS AND METHODS

Species studied and collection data are listed in Table 1. Identification and nomenclature follow Ding Hou (1958). In this treatment, *Gynotroches* is considered to consist of one rather variable species, but material used for this study appeared to represent two very distinct ecotypes or species; differences are noted below. Adequate embryological material was available for only one species in each of the genera *Carallia* (eight species) and *Crossostylis* (six species). Only four of the nine species of *Pellacalyx* were examined, and although complete series of all developmental stages were not available for all four, the data obtained suggest that the genus is probably embryologically uniform.

Material was generally fixed in the field in formalin-acetic acid-60% ethanol (10:5:85 or 5:5:90), but some Brunei collections were fixed in whiskey and formalin (about 10:1). Most material was dehydrated in a tertiary butanol/ethanol series and embedded in paraffin, then sectioned at 8–10 μm and stained with either Heidenhain's iron hematoxylin or safranin followed by fast green (Johansen, 1940). Other material was dehydrated in ethanol, embedded in Polysciences JB-4 resin, sectioned with glass knives at 4–6 μm, and stained with iron hematoxylin.

OBSERVATIONS

Embryological character states are summarized in Tables 2 and 3. Detailed descriptions follow, including notes of occasional variations in character states and some features not always included in embryological summaries.

Carallia borneensis Oliver

Anthers of this species are medifixed, and the connective and the tips of the two halves of the anther are slightly prolonged. The anther consists of four microsporangia. The sporangial wall development is of the "basic" type, that is, having two middle layers, one sharing a common origin with the endothecium; the other, with the tapetum (Fig. 1). Neither the epidermis nor the middle layers persist to anthesis. The endothecial cell walls have very few thick bars of secondary wall (so-called "fibrous" thickenings; Fig. 2). The tapetum is glandular; its cells have two nuclei. Cytokinesis in microspore mother cells is simultaneous, producing tetrahedral tetrads. Anther dehiscence is introrse and occurs by longitudinal slits.

TABLE 2. *Anther and pollen characteristics of tribe Gynotrocheae.*

	Carallia	*Crossostylis*	*Gynotroches*	*Pellacalyx*
Number of microsporangia	4	4	4	4
Anther wall development	basic	basic	monocotyledonous	basic
Epidermis at anthesis	degenerate	persistent	degenerate	persistent, collapsed
Middle layers	degenerate	degenerate	degenerate	degenerate
Endothecial wall thickenings	present	present	present	present
Tapetum	glandular	glandular	glandular	glandular
Tapetal cell nuclei	2	2	2	2
Meiotic cytokinesis	simultaneous	simultaneous or successive	simultaneous or successive	simultaneous or successive
Pollen tetrads	tetrahedral	tetrahedral or decussate	tetrahedral or decussate	tetrahedral or decussate
Pollen nuclei	2	2	2	2

Ovules of *C. borneensis* are bitegmic, and even at early stages of development each integument consists of more than two cell layers (Fig. 3). Early cell divisions in the integuments are irregularly oriented, as would be expected in a multiseriate, parenchymatous structure; by contrast, the anticlinal divisions in the biseriate integuments of other inland Rhizophoraceae are usually uniformly perpendicular to the surface, as would be expected in a structure consisting of two protodermal layers. The outer integument of *C. borneensis* thickens to 7–15 cell layers by the time of anthesis; the inner integument, to about 10 cell layers (Fig. 4). Both integuments contribute to forming the micropyle, and the endostome and exostome are not aligned. The outer integument is vascularized, but the inner integument is not (Fig. 5). The outer epidermis of the inner integument, which forms the prominent exotegmen in other genera, is distinctly differentiated, and a pronounced endothelium is formed as megagametogenesis begins; its cells are densely staining and palisadelike by the two-celled stage of gametogenesis (Fig. 4). At anthesis, the endothelium becomes tanninized and thick-walled.

The archesporial cell divides once periclinally, and the parietal derivative usually divides once anticlinally (Fig. 6). The ovule is thus crassinucellate. A cell wall is formed after meiosis I, and a linear, T-shaped, or irregular tetrad of megaspores is formed. The gametophyte develops from the chalazal megaspore. The observation of two-, four-, and eight-nucleate stages confirmed that gametogenesis occurs according to the *Polygonum*-type pattern. Three antipodal cells are formed, but their nuclei appear condensed or degenerate at anthesis. The mature synergids are pyriform and stain very densely. The polar nuclei are closely appressed but do not fuse to form a secondary nucleus before fertilization. Most of the nucellus, particularly its micropylar half, degenerates during megagametogenesis (Fig. 4).

Fertilization is porogamous, and endosperm formation is initially free-nuclear. Endosperm development and expansion of the fertilized ovule both proceed for some time before embryogenesis begins. The first division of the zygote was roughly transverse in all specimens studied (over 100 seeds in early developmental stages). However, the orientation of the second division was variable. In most cases it was parallel to the axis of the proembryos ("vertical" or longitudinal, although the axis of the proembryo was not usually parallel to the long axis of the ovule), but in many other specimens it was oblique (Figs. 7, 8). Although the basal cell subsequently divides transversely and these derivatives divide both transversely and longitudinally, none of the resultant cells contribute to the embryo proper. Thus, allowing for a slight relaxation of the definition of onagrad-type embryogenesis to accommodate the variability in orientation of the plane of division of the terminal cell, embryogenesis in *C. borneensis* can be described as being fundamentally of this type. During the early globular stage (proembryo consisting of up to about 20 cells in median longitudinal section), both the basal cell of the suspensor and the cells closest to the embryo proper divide longitudinally, producing a mostly multiseriate suspensor, sometimes with a uniseriate section in the middle (Fig. 8).

The mature embryo consists of two laminar cotyledons and a straight hypocotyl, this making up about two-thirds of the length of the embryo. The axial vascular cylinder is medullated throughout its length. The embryo is green and enveloped by abundant endosperm. The seed coat is mostly testal and 20–50 cell layers thick; its surface is bullate

TABLE 3. *Ovule and seed characteristics of tribe Gynotrocheae. See text for additional details.*

	Carallia	*Crossostylis*	*Gynotroches*	*Pellacalyx*
Inner integument cell layers at inception/anthesis	2–3/many[1]	2/20	2/5–6	2/8–10
Outer integument cell layers at inception/anthesis	3 or more/many[1]	2–3/4–5[2]	2/2	2/2
Integument vascularization	outer only[3]	none	none	none
Micropyle	diplostomic	diplostomic	diplostomic	diplostomic
Endothelium	present	present	present	present
Archesporial cell(s)	several	several	1	1
Megasporangium	crassinucellate	crassinucellate	tenuinucellate	tenuinucellate
Megaspore tetrad	T-shaped or linear	linear	T-shaped	linear
Megagametogenesis	chalazal-monosporic	chalazal-monosporic	chalazal-monosporic	chalazal-monosporic
Antipodals at anthesis	condensed	condensed	persistent	persistent
Synergid shape	pyriform	pyriform	pyriform	pyriform
Secondary nucleus	not formed	not formed	not formed	not formed
Endosperm development	free-nuclear	—	free-nuclear	free-nuclear
Endosperm transfer cells	absent	—	absent	absent
Embryogenesis	variable	—	variable	variable
Exotegmen	unsclerified	—	sclerified	sclerified
Cotyledons	2, foliaceous	—	2, foliaceous	2, foliaceous

[1] Integuments of species other than *C. borneensis* are thinner at maturity and possibly also at inception.
[2] Strictly biseriate outer integument was never observed, possibly because periclinal divisions begin very early.
[3] Presently known only in *C. borneensis*; not found in *C. brachiata* or *C. eugenioidea*.

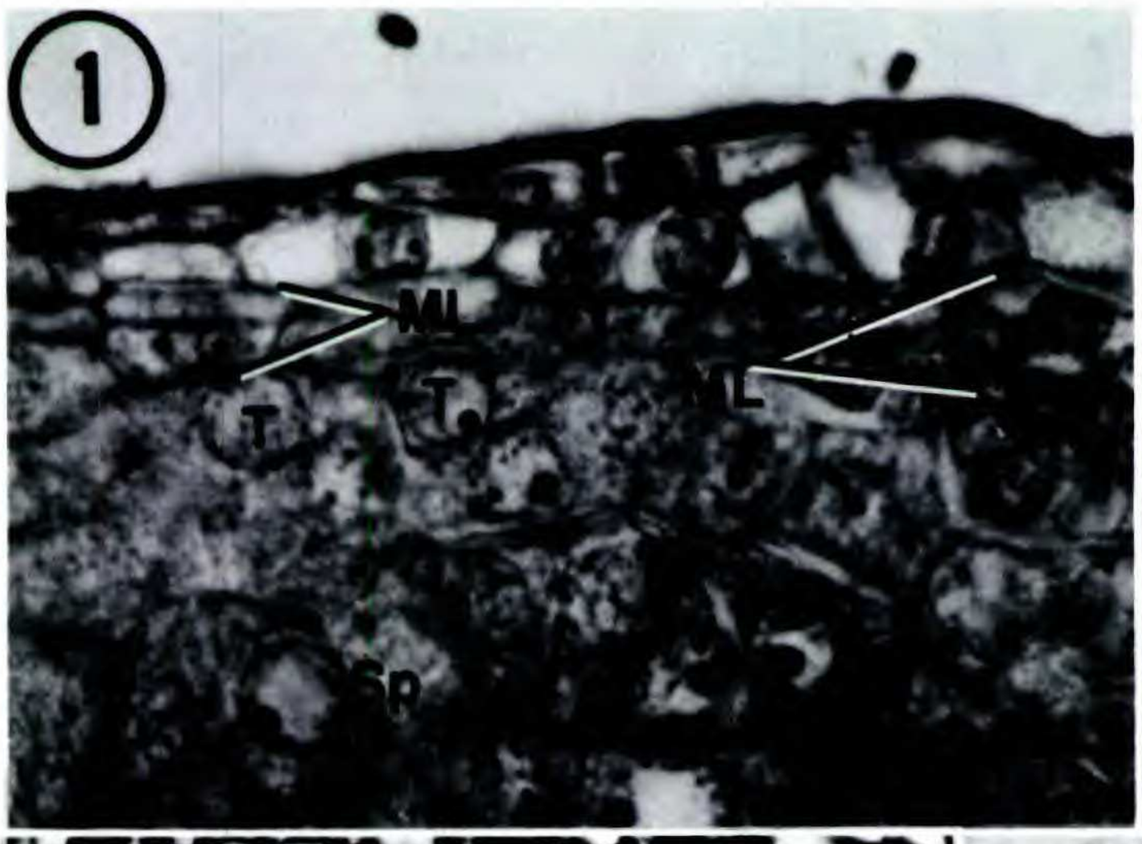
due to localized growth of hypodermal parenchyma and expansion of the overlying epidermis (Fig. 9). Although the exotegmen is a distinctive cell layer in the unfertilized ovule, it does not persist to form a prominent sclerified layer in the mature seed, as in other inland genera of Rhizophoraceae; instead, the tegmen gradually degenerates and/or is crushed during later seed development.

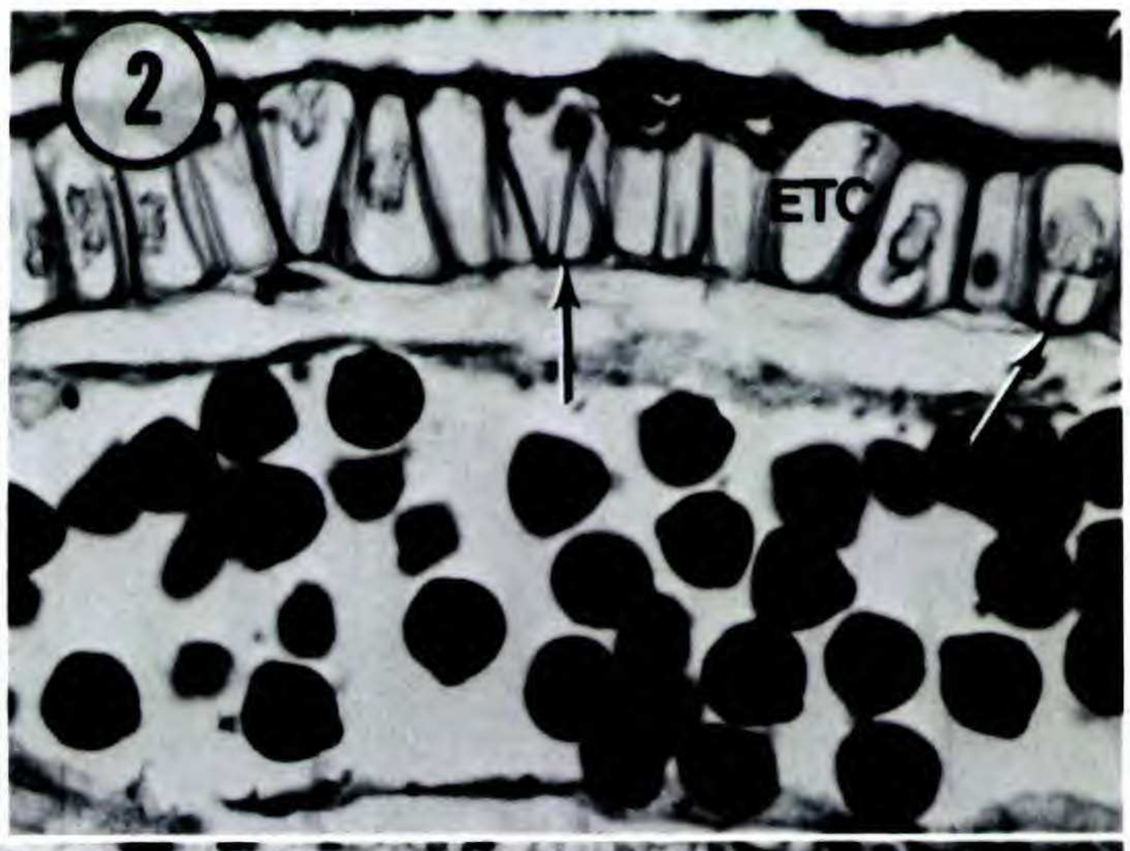
Crossostylis grandiflora Brongn. & Gris.

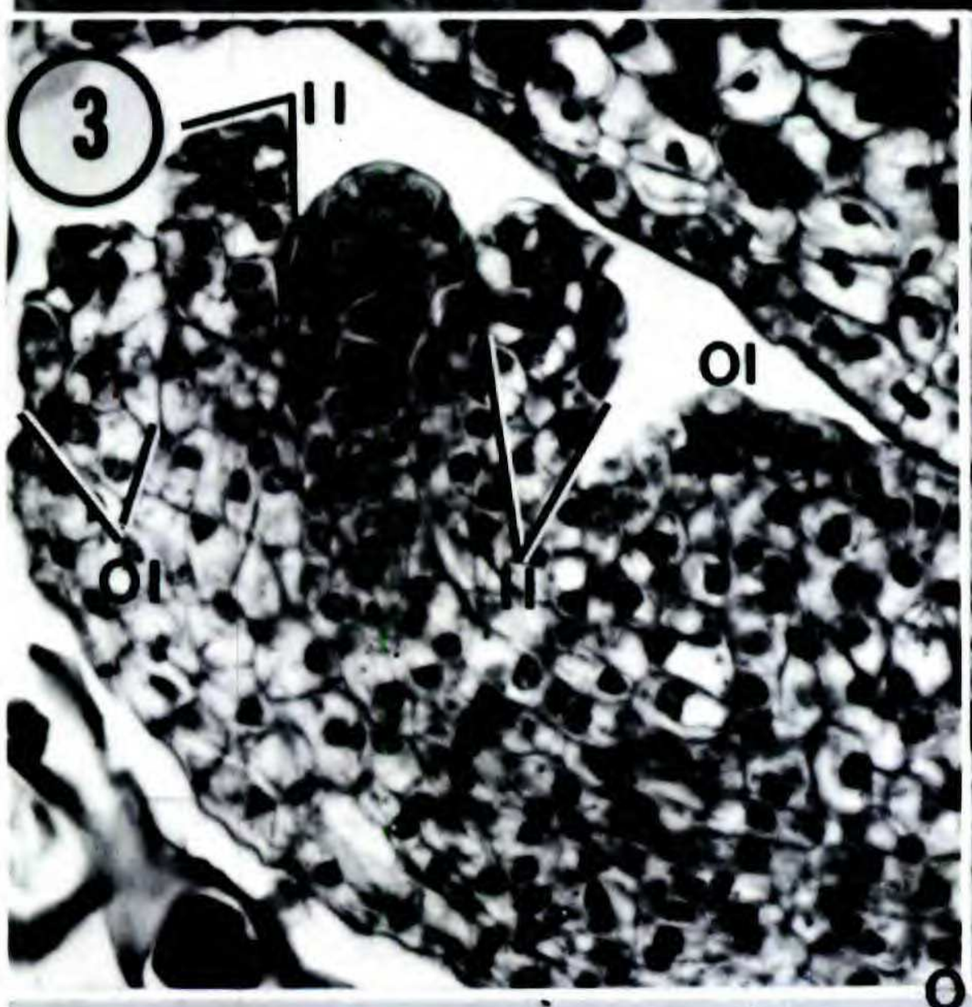
The anther consists of four microsporangia. Periclinal divisions in the outer parietal cell layer produce the endothecium and a primary middle layer, which in turn divides to form two cell layers. Later, the inner parietal cells divide, producing the tapetum and a third middle layer (Fig. 10). Thus, anther wall development follows the basic type. The middle layers degenerate during microsporogenesis. The epidermis persists to anthesis, although tannins are deposited in its cells. A few thick bars of secondary wall are formed by the endothecial cells (Fig. 11). As in other Rhizophoraceae, the tapetum is glandular, and its cells are binucleate. However, unlike other genera, the tapetal cytoplasm in *C. grandiflora* stains more densely with hematoxylin and may include several to many unstained globules of uncertain composition.

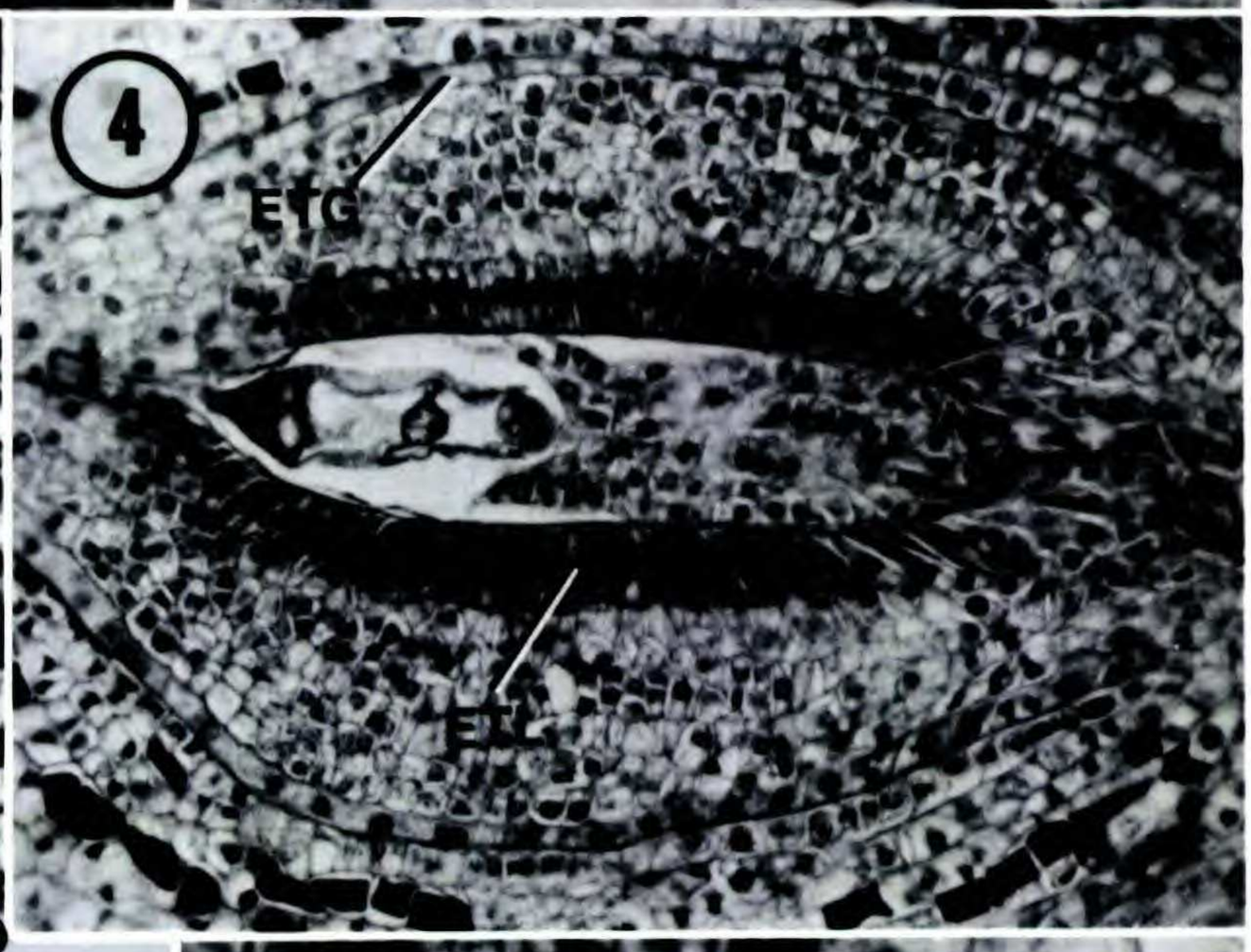
Microsporogenesis occurs by both simultaneous and successive cytokinesis, but most cytokineses in a single microsporangium occur by only one pattern (Figs. 12, 13). Although any one cell in which simultaneous nuclear division (but not wall formation) has occurred may resemble a cell at the end of the nuclear division of meiosis I, careful examination at various focal levels of hundreds of meiotic figures in many serial sections of several different flowers revealed virtually no cells with tetrahedrally arranged nuclei in "successive" thecae, such as the one shown in Figure 13, and revealed virtually no binucleate cells or portions of cells in "simultaneous" thecae, such as that shown in Figure 12. The very large number of observations makes us confident that this conclusion is not due to orientation of the cells relative to the plane of sectioning, but rather reflects actual variability in the meiotic division pattern. In successively dividing cells, wall formation occurs after meiosis I but is not documented here for this genus (see also Fig. 25, *Pellacalyx*). Pollen tetrads of *C. grandiflora* are either tetrahedral or decussate. The mature anthers are strongly reniform, even semicircular, and dehisce introrsely by longitudinal slits.

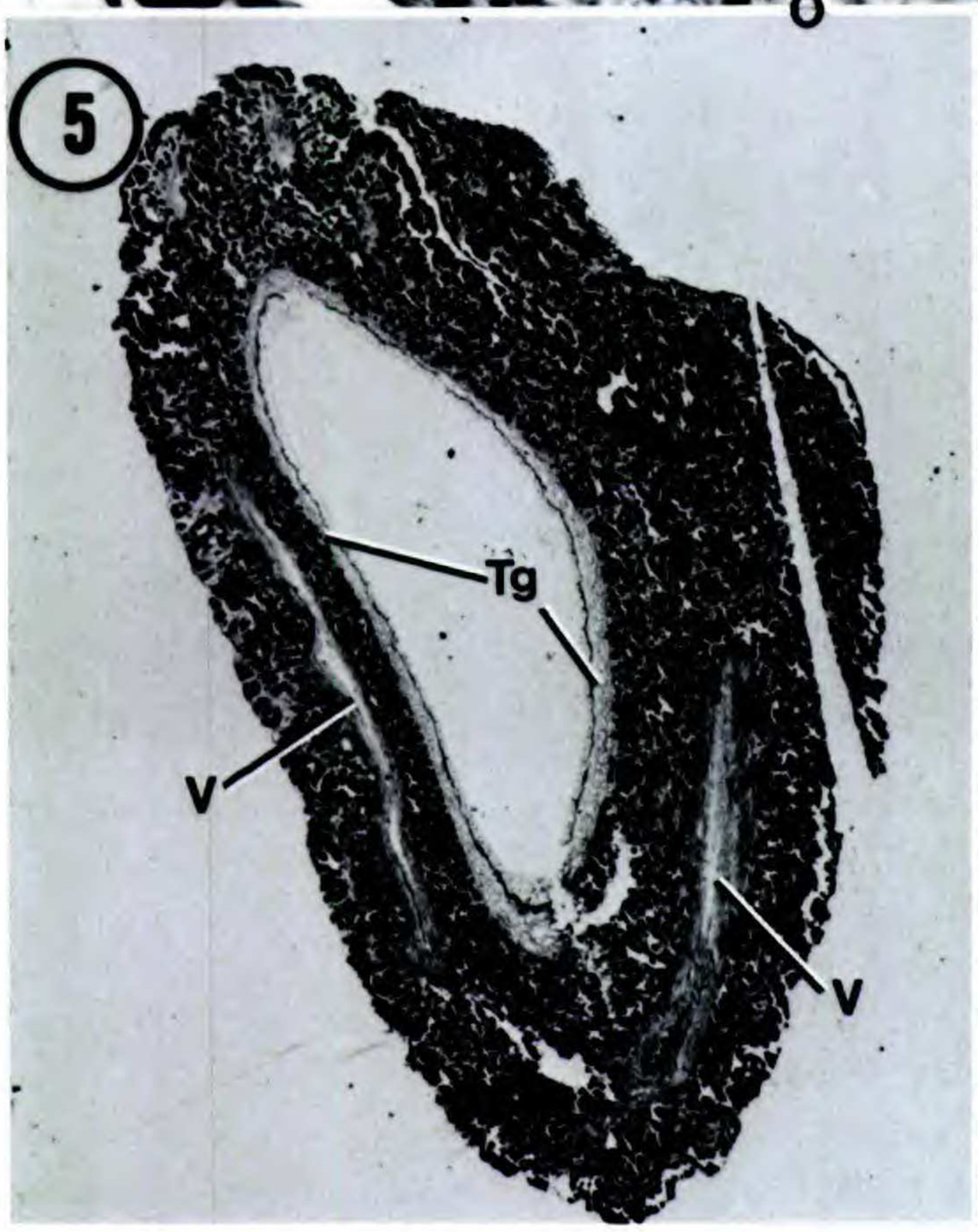
The ovule is bitegmic. At inception and during early development, the inner integument is biseriate (Fig. 15). Later, numerous peripheral divi-

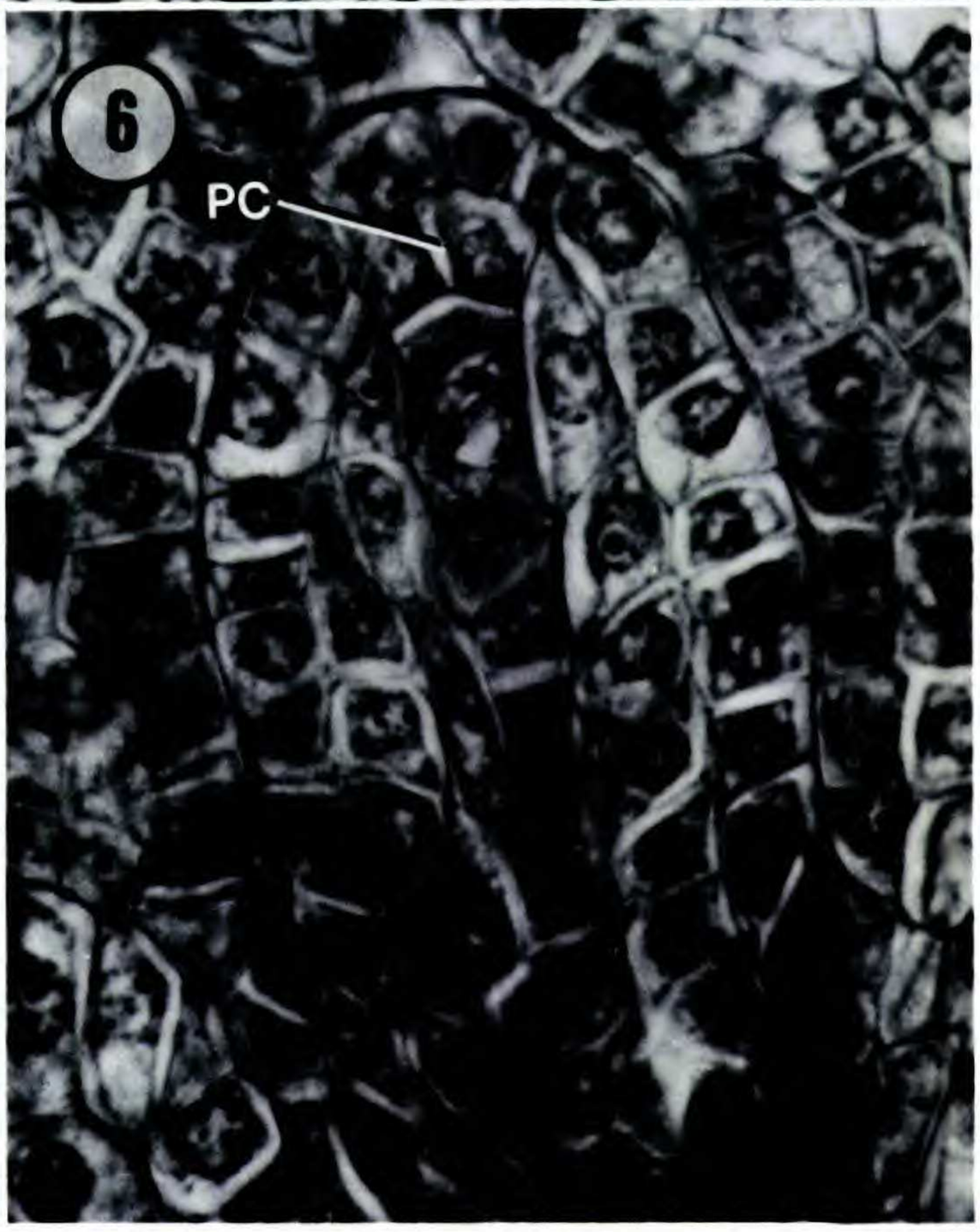




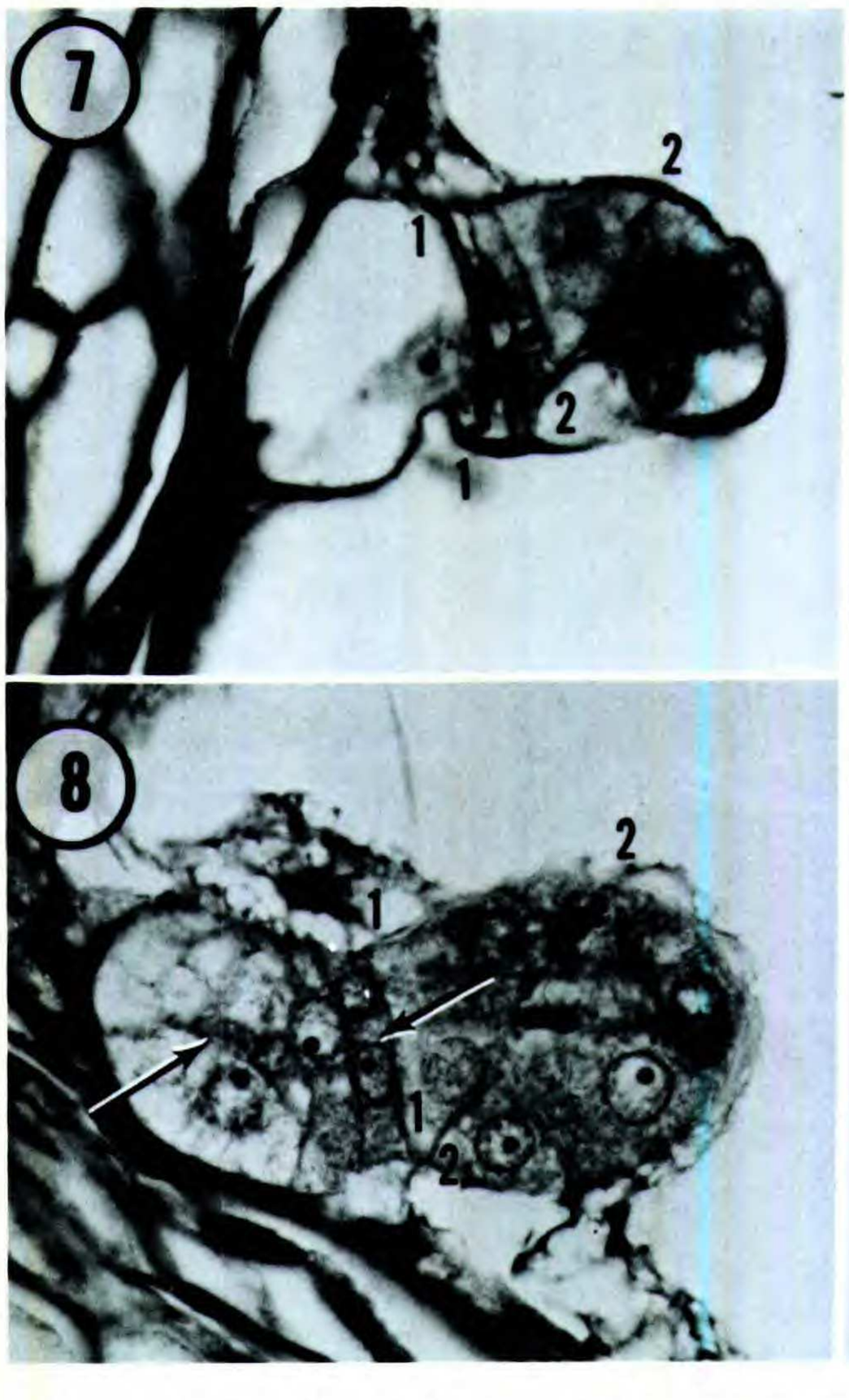








FIGURES 1–6. Carallia borneensis. — *1. Basic-type anther wall development. ML, middle layers; Sp, sporogenous cells; T, tapetum. ×1,140.—2. Anther just before anthesis; of the wall layers, only the endothecium (ETC) persists. Longitudinal radial walls have at most one wall thickening (arrows). ×455.—3. Ovule, early devel-*



FIGURES 7–9. Carallia borneensis, *proembryo and seed coat.—7. Proembryo showing transverse first division (1) and oblique second division (2). ×635.—8. Later proembryo showing longitudinal divisions in suspensor cells (arrows). ×650.—9. Developing seed coat. Bumps on its surface are formed by growth of both epidermis and hypodermis of testa (Ts). Some of tegmen (Tg) persists at this stage but degenerates later; its outer epidermis does not sclerify. ×120.*

sions occur, and it becomes about 20 cells thick by the time of anthesis (Fig. 16). The exotegmen and endothelium are sharply differentiated throughout megagametogenesis. The outer integument is probably biseriate at its inception, although this stage was not observed. In the premeiotic stages that we studied in which the outer integument (or at least its abaxial portion) was present, its basal half was triseriate and the distal portion was biseriate. During the remainder of ovule development very few periclinal divisions occur in the outer integument, and at anthesis it is only about four cell layers thick. Its outer epidermal cells expand considerably and are filled with tannin.

Gynotroches axillaris Blume

Collections used for this study came from two sites in southern Sarawak. Study of plants from these and a number of other sites suggests that at least two ecotypes, probably constituting distinct species, are found in this region. This lends strong support to the suggestion by Ding Hou (1958) that, with further study, this highly variable and widely

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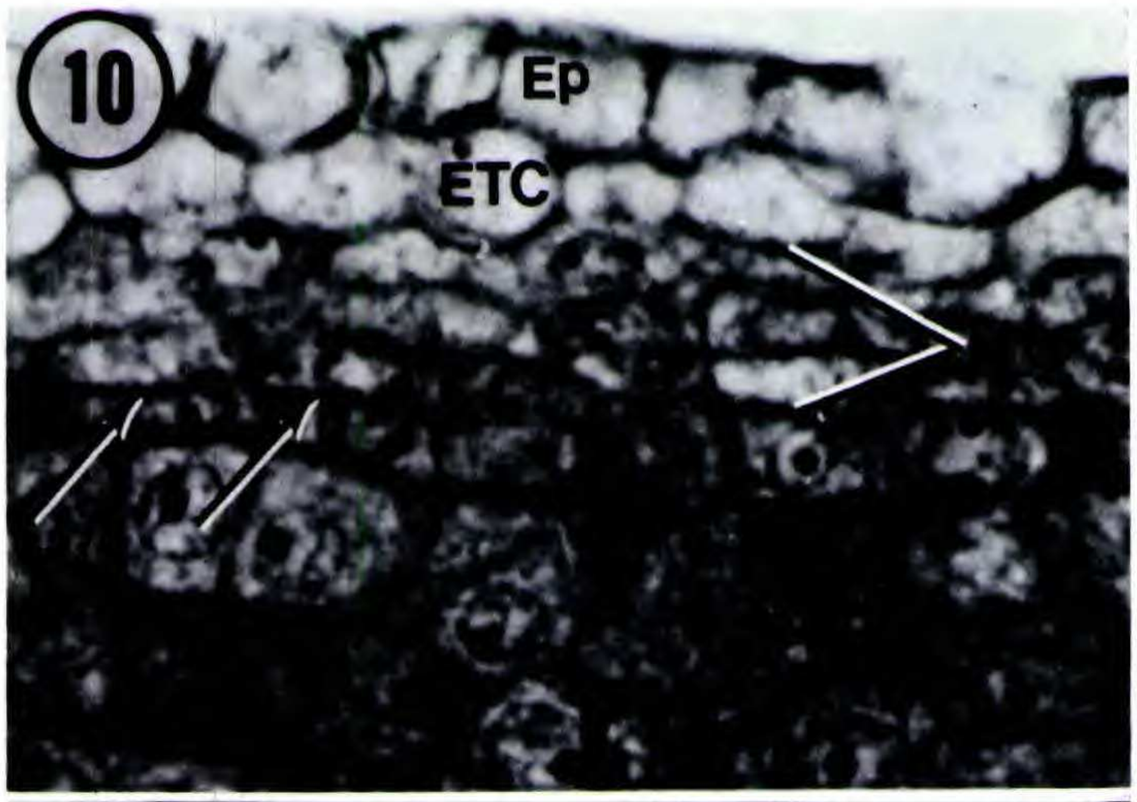
opmental stage. Adaxial portion of inner integument (II at center) is three cell layers thick; abaxial portion (II above left) is partly biseriate. Outer integument is not yet developed adaxially (OI at right indicates the primordium), but its abaxial portion (OI at left) is multiseriate. ×445.—4. Ovule shortly before anthesis. Megagametophyte had eight nuclei, some in adjacent section; note also degeneration of nucellus and presence of endothelium (ETL) and differentiated outer epidermis (ETG) in inner integument. ×285.—5. Developing seed, showing vascularization of outer integument (V). In this very immature seed, some inner integument or tegmen persists (Tg), but this degenerates as the seed develops. ×20.—6. Nucellus with megaspore mother cell (MMC) separated from epidermis by two parietal cells (PC). ×1,140.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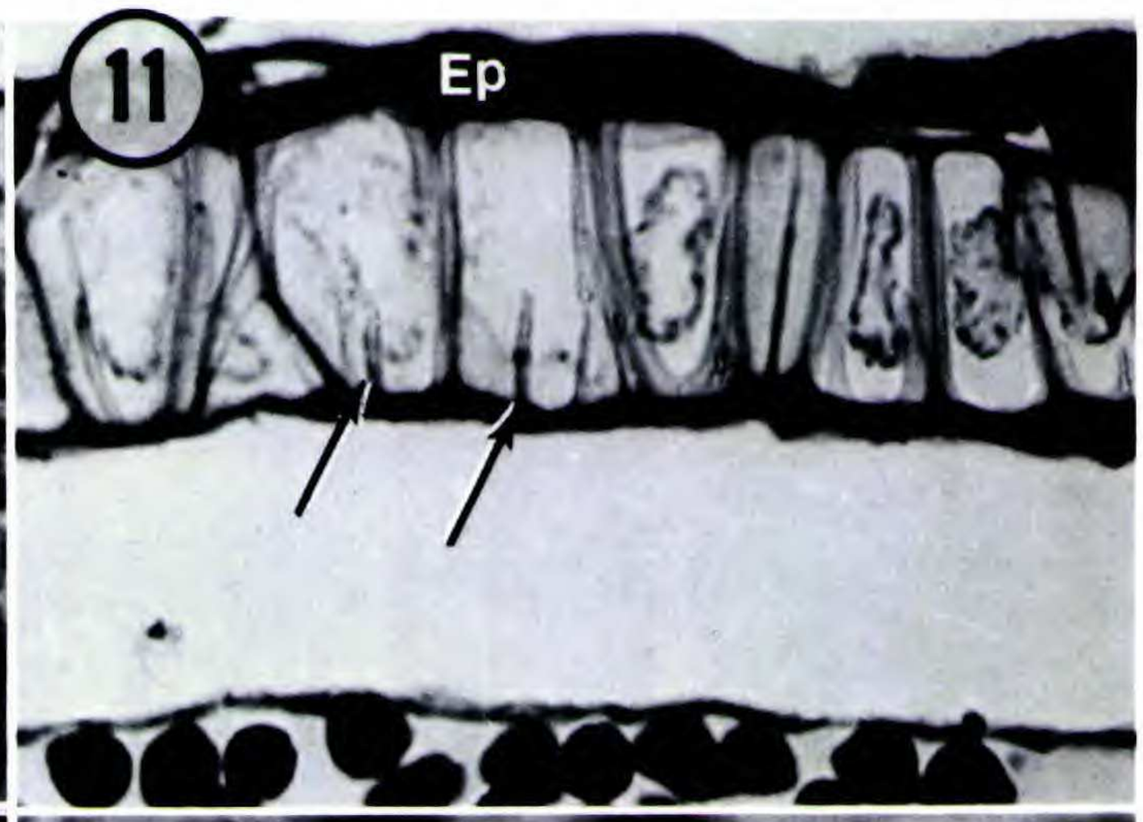
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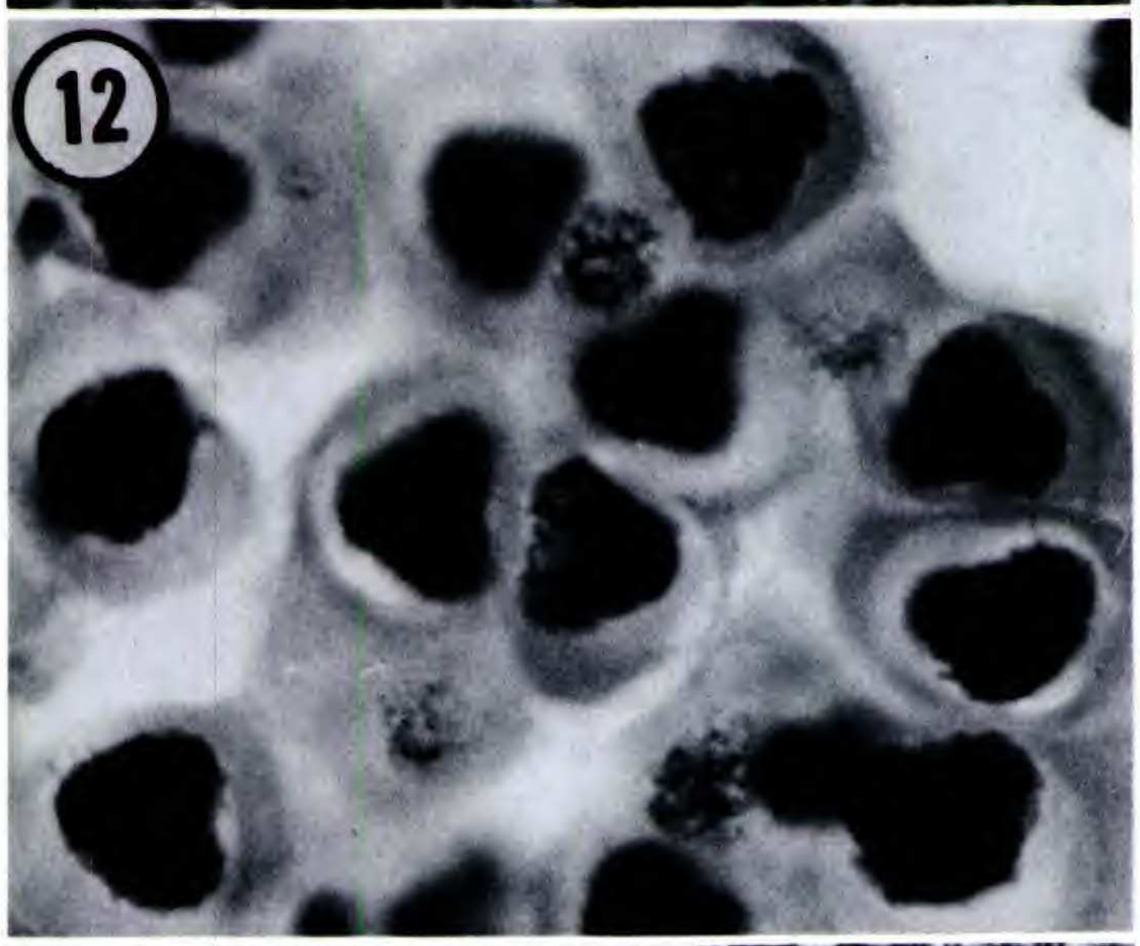
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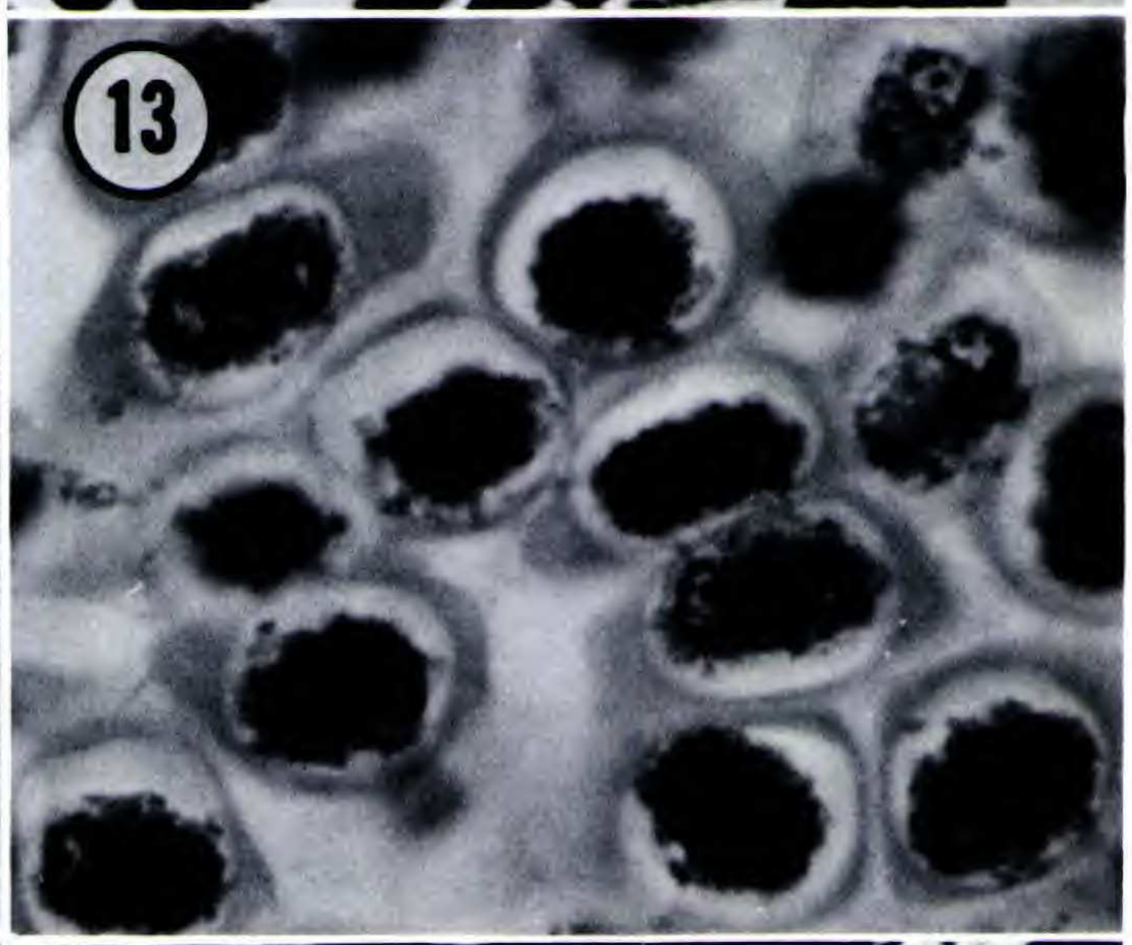
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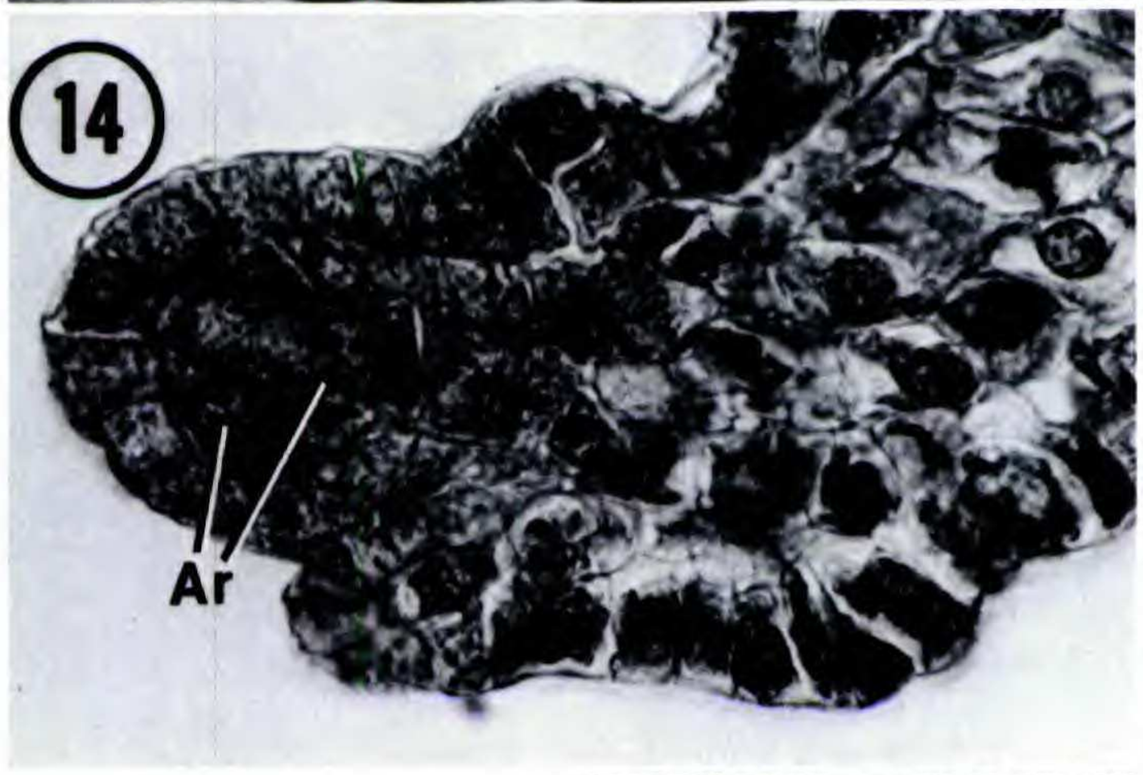
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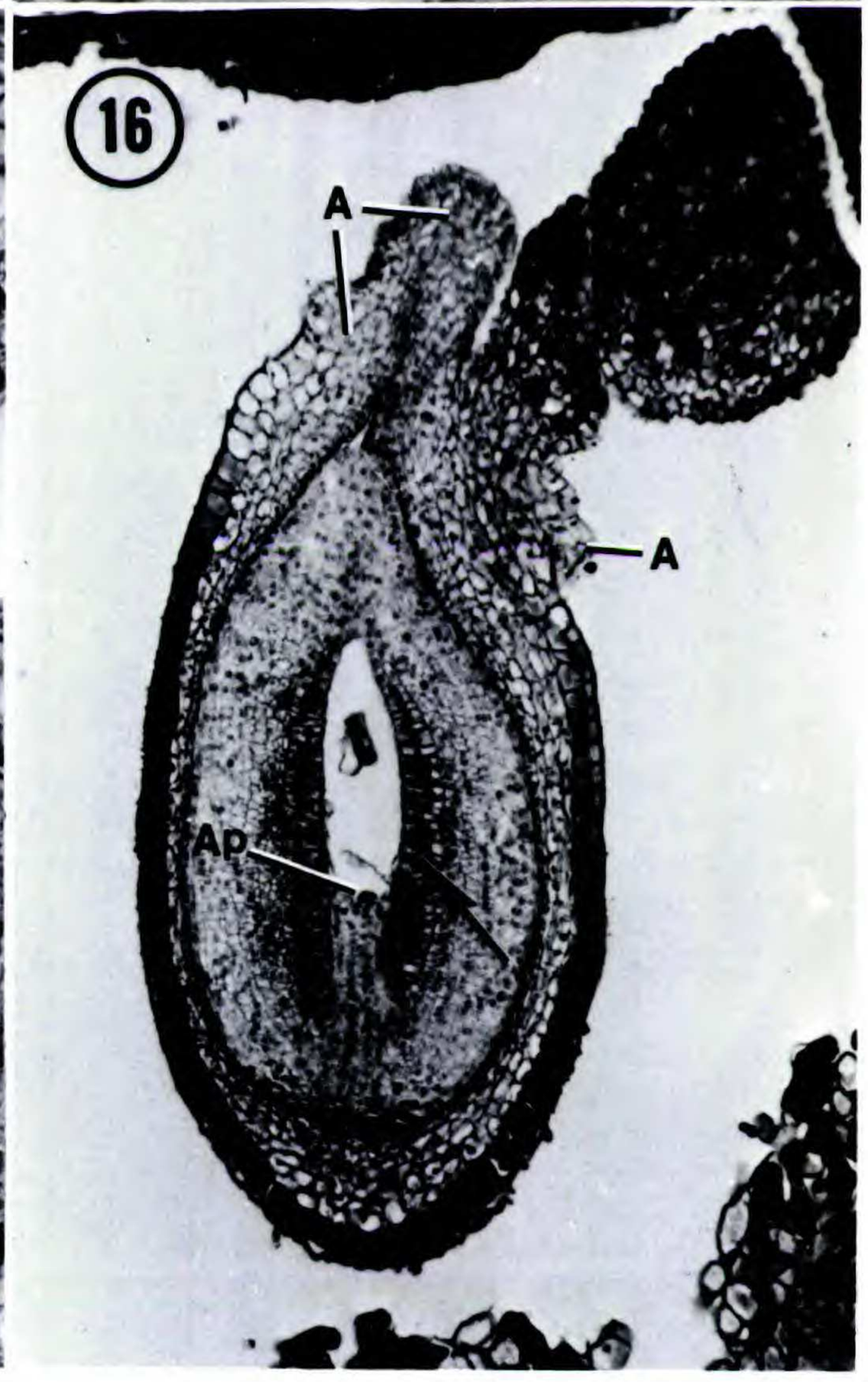
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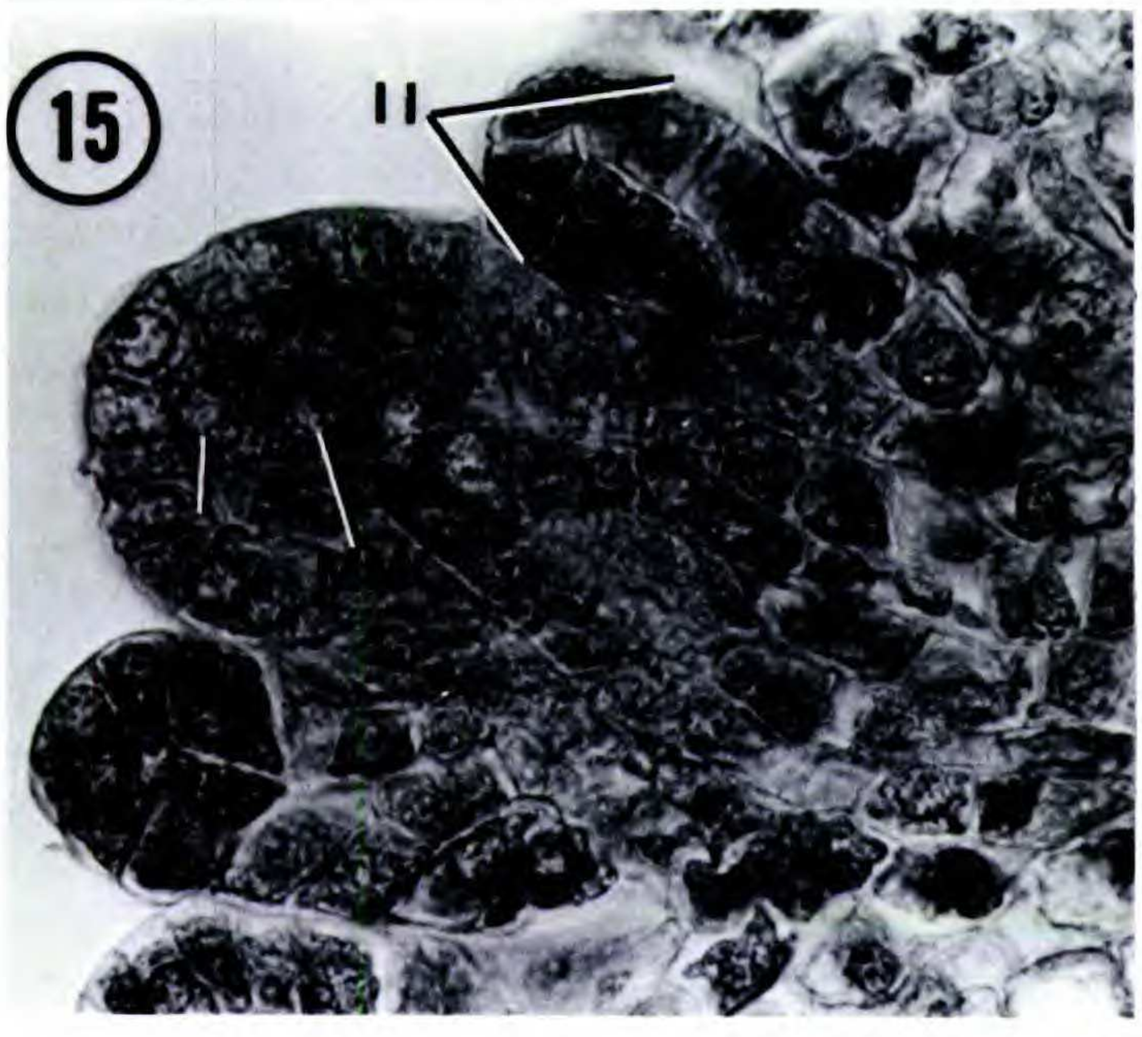
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distributed genus will be amenable to subdivision into several distinct species. Accordingly, brief descriptions of the taxa collected are given so that future readers will be able to ascertain to which species the embryological data pertain. Trees found in secondary vegetation on low altitude kerangas sites or peat swamps had smooth leaves about 6–9 cm long and reddish fruits about 4 mm in diameter at maturity, usually largely covered by a cracked periderm. Trees found along the banks of small streams in hill forest at 50–200 m elevation had bullate leaves 20–25 cm long and yellow, noncorky fruits at least 5–6 mm in diameter at maturity. In addition to these nonoverlapping color and size differences, the trees had very different habits and leaf colors. Both species produce aerial stilt roots, which are more numerous on trees growing in very swampy sites. Both taxa observed appeared to be dioecious. Absence of developing or mature fruits on trees bearing male flowers suggests that the apparent dioecy did not result merely from a temporal separation of the two types of flowers. Almost all of the embryological data reported herein were derived from study of specimens collected in the hill forest.

The anther of *Gynotroches* consists of four microsporangia. Only one middle layer is present between the endothecium and tapetum; because this layer shares its origin with the tapetum, anther wall development is of the "monocotyledonous" type (Fig. 17). The tapetum is glandular; its cells have two nuclei. In some microsporangia, meiotic cytokinesis was successive, producing isobilateral or decussate tetrads (Fig. 18), but in others, even within the same stamen, simultaneous cytokinesis occurred, producing tetrahedral tetrads (Fig. 19). As with *Crossostylis* (see above), numerous meiotic figures were examined to insure that we were not misled by an artifact of the plane of sectioning, although only a few cells are shown in the figures. Mature pollen grains in male flowers are binucleate. In female flowers, the anther as a whole develops more or less normally, having at least a partially functional tapetum and an endothecium with secondary wall thickenings (Fig. 20). Meiosis also occurs, forming numerous microspores, but the normal sporopollenin wall does not form around them (Fig. 20).

Functional ovules of *Gynotroches* are bitegmic and anatropous. Early in development, both integuments are two cell layers thick (Fig. 21). The inner integument thickens to 5–6 cell layers by the time of anthesis, but the outer integument remains only two cell layers thick in most places, except for its expanded micropylar portion (Fig. 22). A funicular (raphe) vascular bundle extends along the adaxial side of the outer integument, but neither integument proper is vascularized. An endothelium is formed early in megagametogenesis (Fig. 22). The micropylar half of the nucellus degenerates before gametogenesis is complete. The micropyle is quite short and is formed by both integuments. Occasionally, however, extension of the inner integument makes the micropyle endostomic. The single archesporial cell functions directly as the megaspore mother cell; thus, the ovule is tenuinucellate (Fig. 21). A cell wall forms after meiosis I, and the megaspore tetrad is usually T-shaped. The chalazal megaspore forms a normal 8-nucleate megagametophyte via *Polygonum*-type development. The synergids are large and pyriform, and the polar nuclei do not fuse to form a secondary nucleus. Antipodals are formed and persist to anthesis (Fig. 22).

In male flowers, early ovule development is more or less similar to that in female flowers, although the nonfunctional ovules are somewhat smaller and irregularly shaped compared with the fertile ones of female flowers, primarily due to irregularities in integument development (Fig. 23). A normal micropyle can rarely be seen. No obvious irregularities in megasporogensis were noted in the nonfunctional ovules, and megagametogenesis may either proceed normally through at least the four-nucleate stage (Fig. 23) or may be somewhat disordered. Gynoecial development is also modified in

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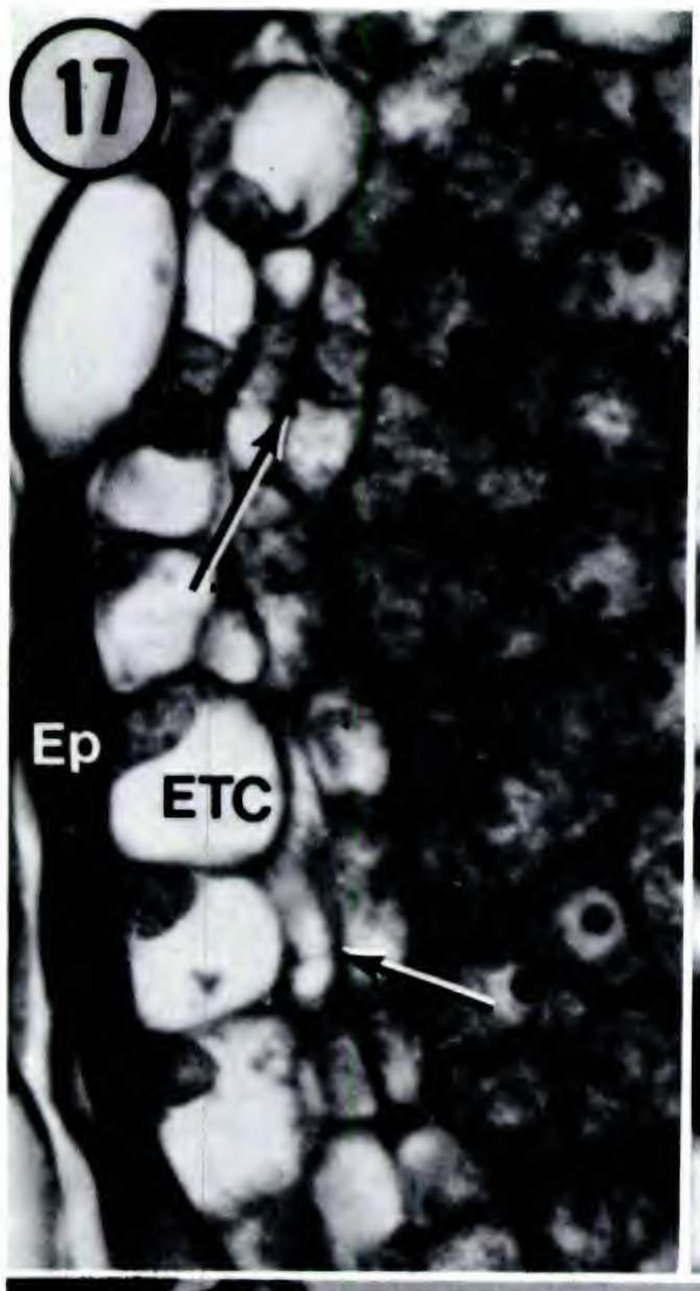
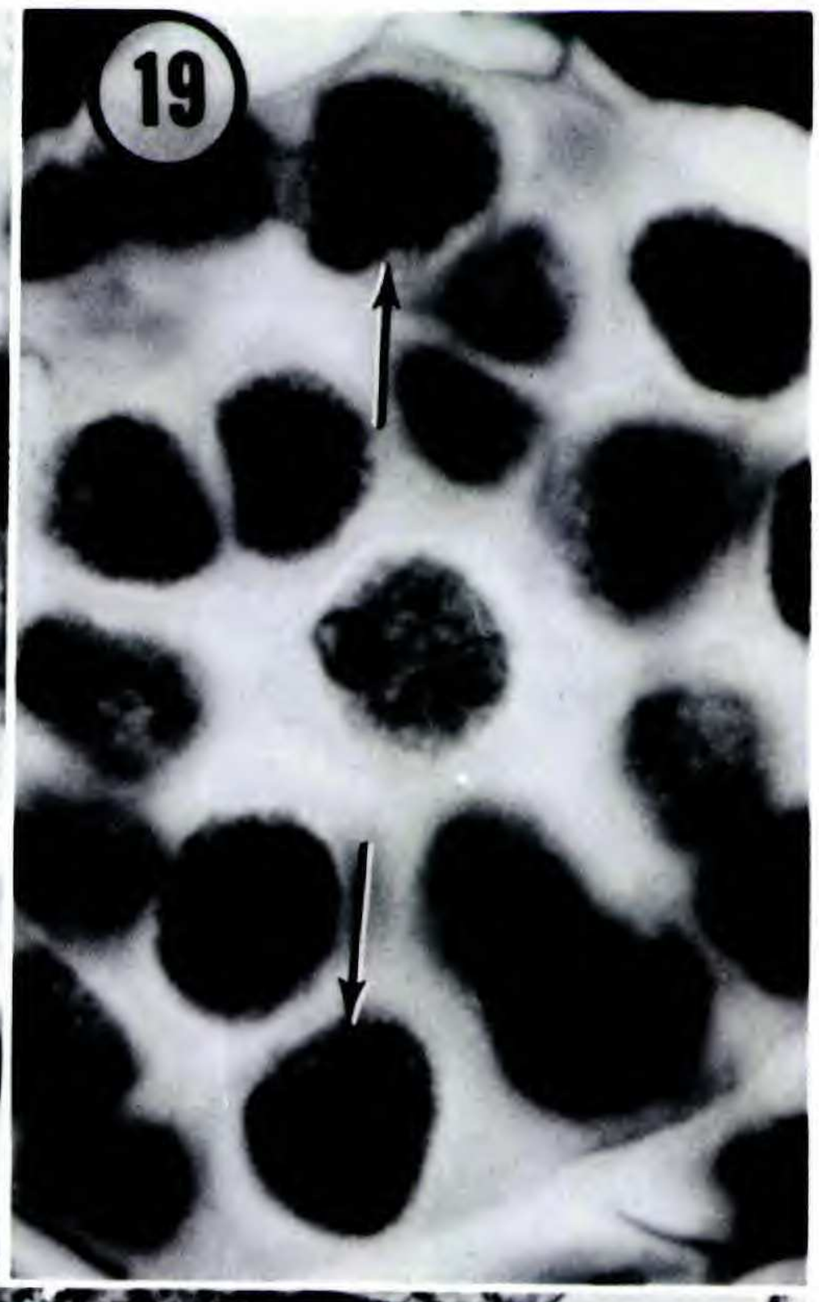
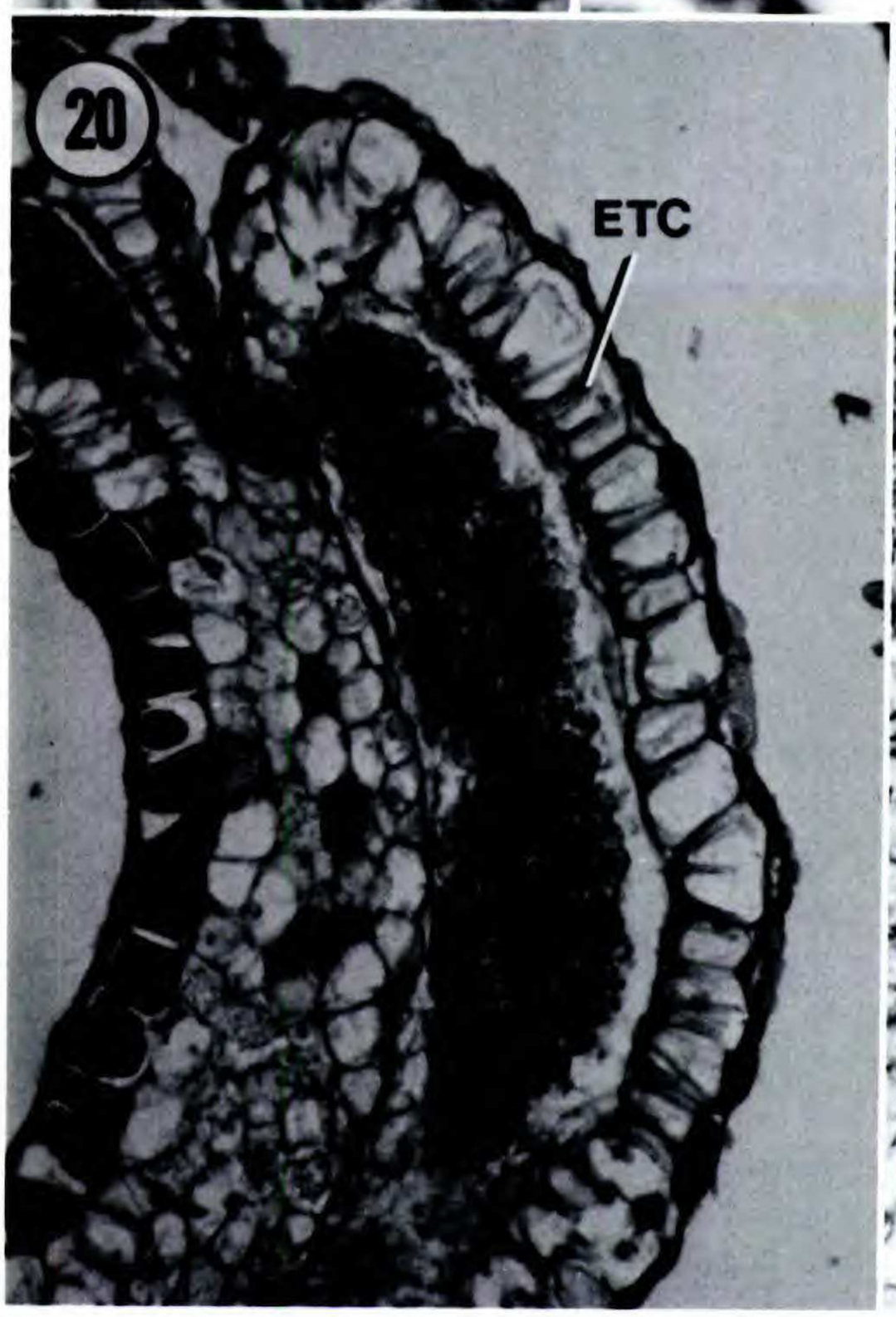
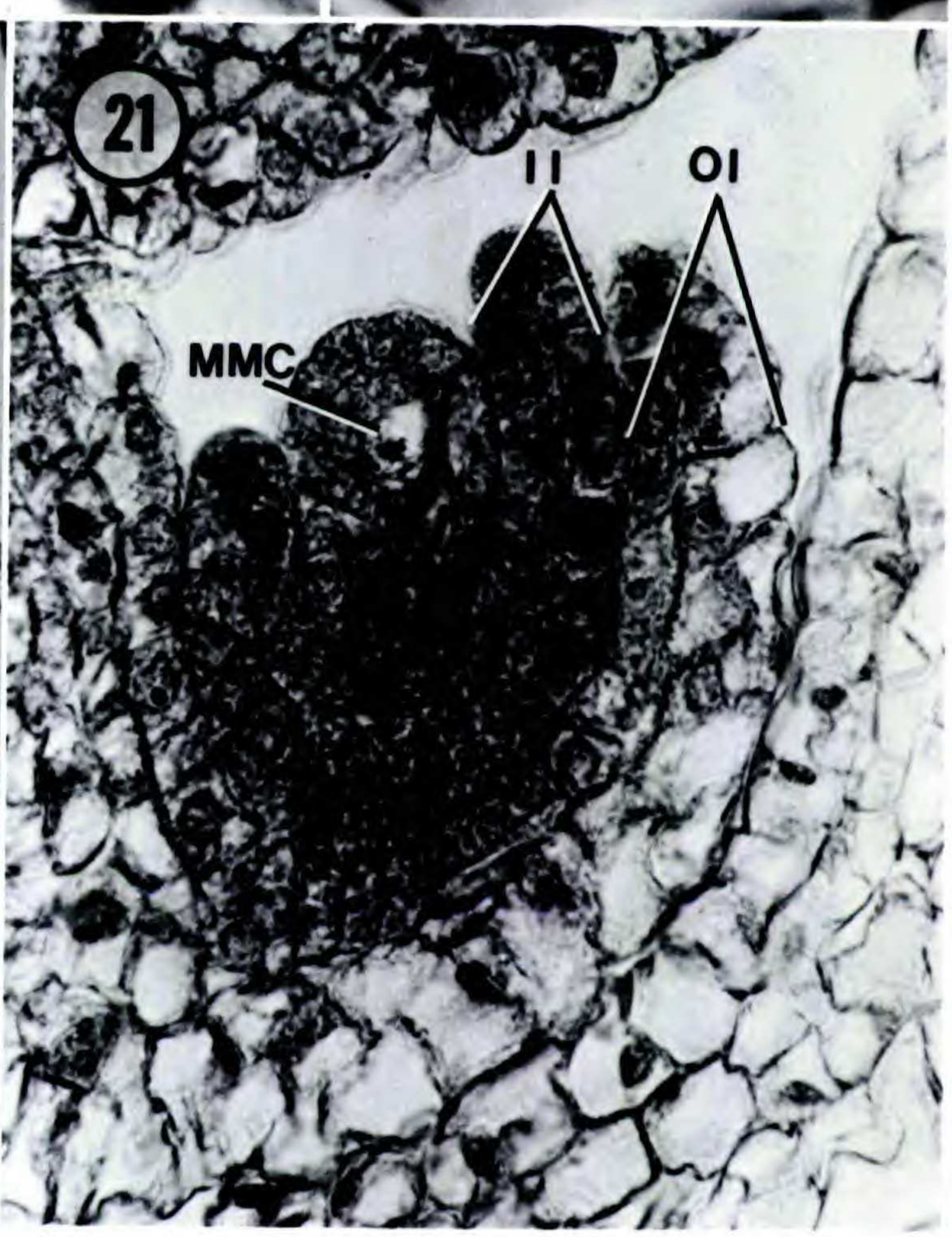
FIGURES 10–16. Crossostylis grandiflora.—*10. Developing anther wall. Typically, at least three middle layers form (ML); periclinal divisions of inner parietal cells are seen here (arrows). Ep, epidermis; ETC, endothecium.* ×*1,140.*—*11. Mature anther wall. Epidermis (Ep) persists, and radial walls of endothecial cells have one or two secondary wall thickenings (arrows).* ×*265.*—*12. Microsporogenesis in a theca where most meiotic divisions were simultaneous.* ×*1,140*—*13. Meiosis I in a theca where most divisions were successive.* ×*1,140.*—*14. Early ovule with at least two archesporial cells (Ar).* ×*800.*—*15. Later ovule, in which the archesporial cell has divided periclinally to form a megaspore mother cell (MMC) and a parietal cell (PC).* ×*800.*—*16. Ovule at anthesis. Inner integument has thickened considerably; outer integument, only slightly, to about four cell layers. Endothelium is prominent (arrow), and aril primordium (A) has begun to develop. Ap, condensed antipodals.* ×*100.*

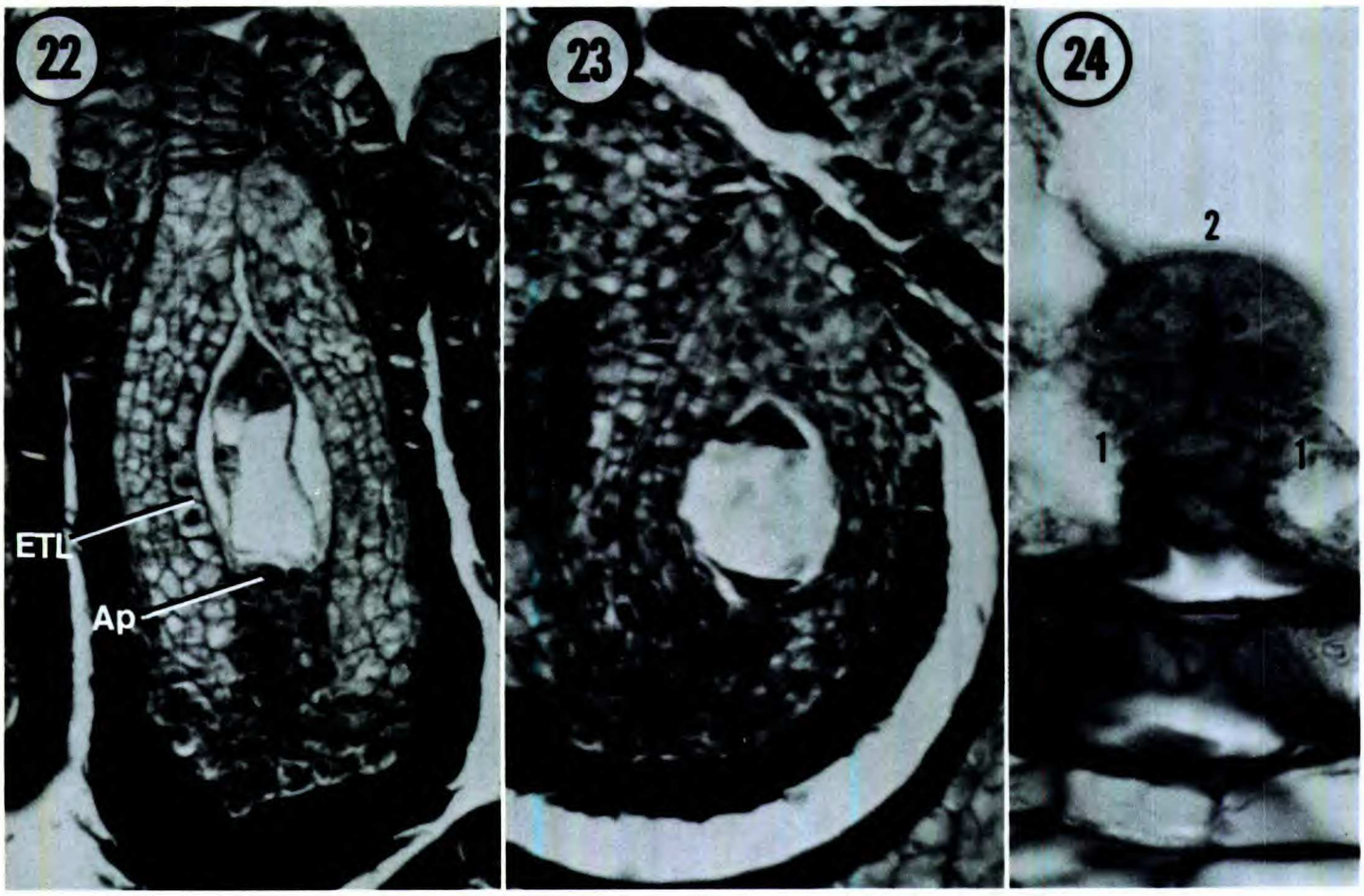








FIGURES 17–21. Gynotroches axillaris, *anther and early ovule.—17. Monocotyledonous-type development of anther wall; arrows indicate divisions forming the tapetum and single middle layer. ×1,060. Ep, epidermis; ETC, endothecium.—18. Meiosis I in a microsporangium where most divisions were successive. ×1,500.—19. Microsporogenesis in a theca where most meiotic cytokineses were simultaneous. Arrows indicate cells with tetrahedrally arranged nuclei, just prior to wall formation. ×1,500.—20. Anther of female flower at anthesis. Normal endothecium is formed, but pollen development is arrested before deposition of sporopollenin wall, which would stain darkly in safranin (used here). ×310.—21. Developing tenuinucellate ovule; archesporial cell functions as megaspore mother cell (MMC) without first dividing periclinally. Both inner (II) and outer integuments (OI) consist of two cell layers. ×900.*



FIGURES 22–24. Gynotroches axillaris, *mature ovule and proembryo.—22. Functional ovule from female flower at anthesis. Most of nucellus has degenerated; endothelium (ETL) is only slightly differentiated. Antipodals persist (Ap). ×425.—23. Sterile ovule from male flower at anthesis. Megagametogenesis has proceeded to the four-celled stage, but ovule morphology is abnormal; micropyle was not evident in this or adjacent sections. ×425.—24. Proembryo formed by classic onagrad-type embryogenesis, which predominates in this genus. 1, first division; 2, second division. ×1,060.*

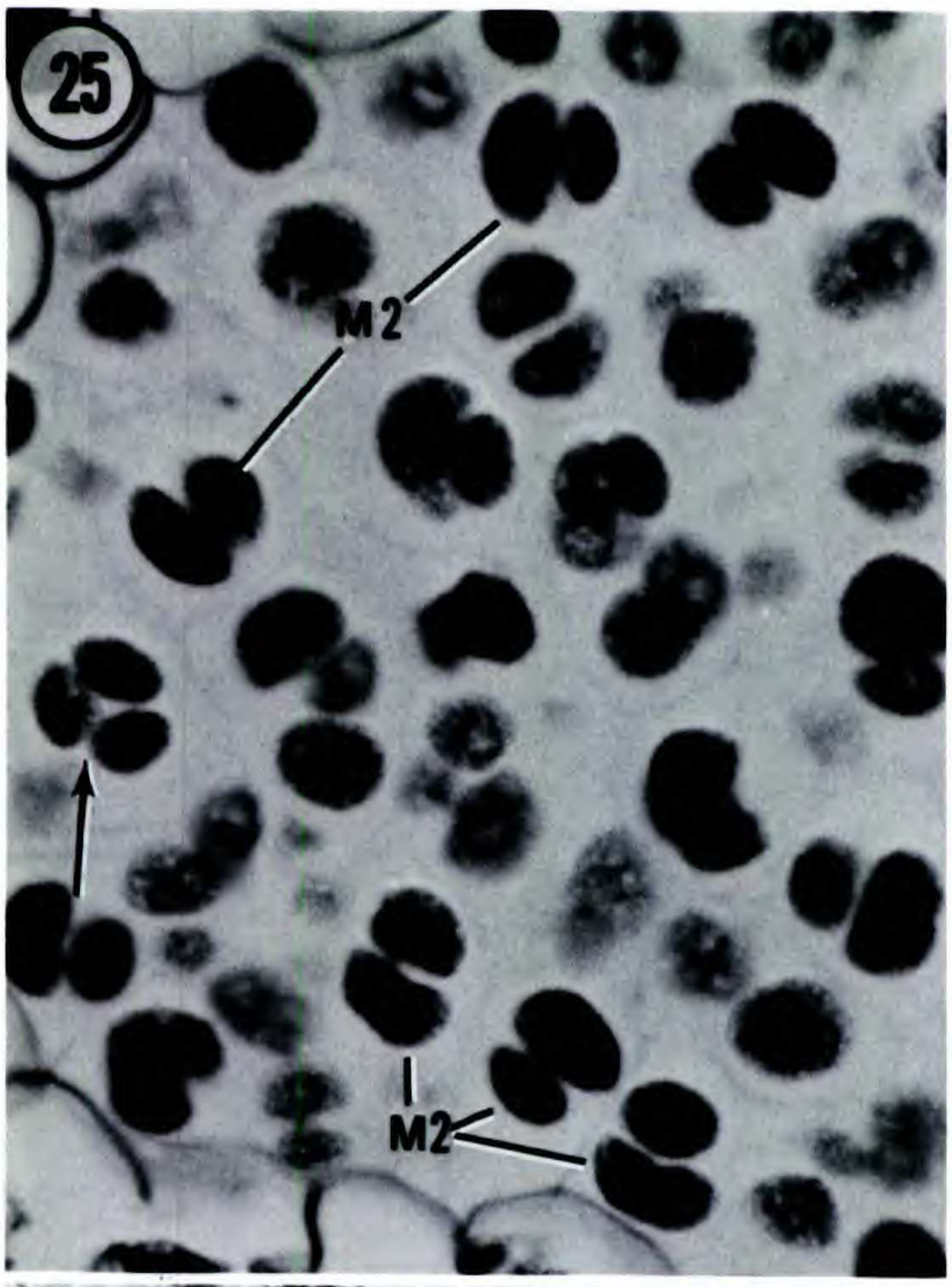
male flowers. In particular, the style may become necrotic or may elongate abnormally, with the stigmatic lobes remaining inserted at its base (Juncosa, pers. obs.). Further details of floral development will appear separately.

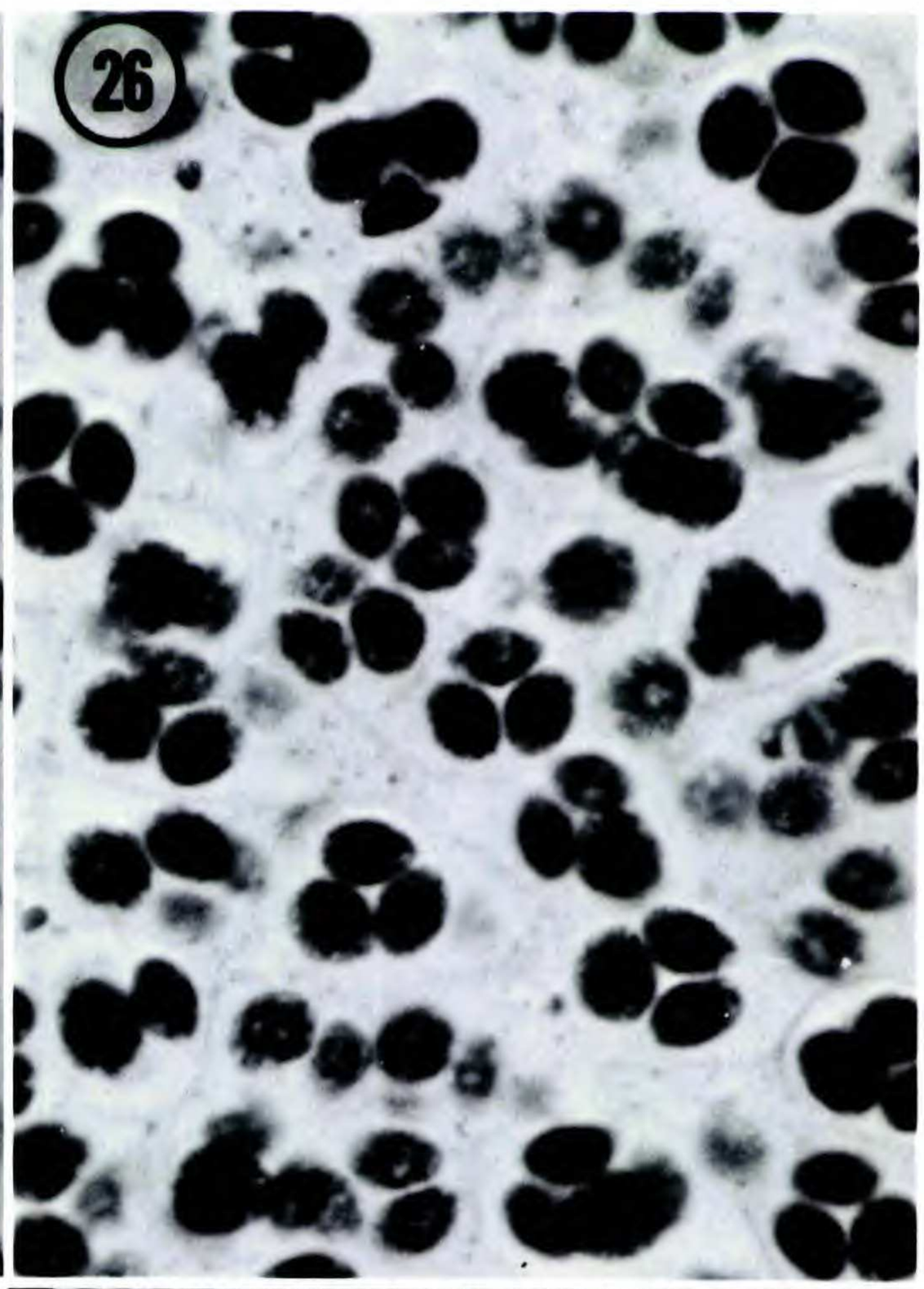
In fertilized ovules of female flowers, both cell layers of the outer integument persist and form a distinctive testa, in which the outer epidermal cells are very large and tanniniferous, and the inner epidermal cells are small and compact, with prominent nuclei and dense cytoplasm. This latter layer of cells may play an important role in controlling the process of imbibition. The exotegmen is very prominent and sclerified; radial elongation of some of its cells makes its outline undulate. Several internal cell layers of the inner integument also persist but remain unspecialized. Mature endosperm cells contain many large inclusions of undetermined composition. These inclusions stain weakly with safranin and are not birefringent. As in other Rhizophoraceae, the embryogenetic cell division pattern in *Gynotroches* is variable. However, in this genus, the overwhelming majority of proembryos observed had developed according to the typical onagrad-type pattern (Fig. 24), and only a few exhibited an oblique division of the terminal cell. The mature embryo is straight, and both the hypocotyl and cotyledons are well developed; they are of roughly equal length. The provascular cylinder in the radicle is not medullated.

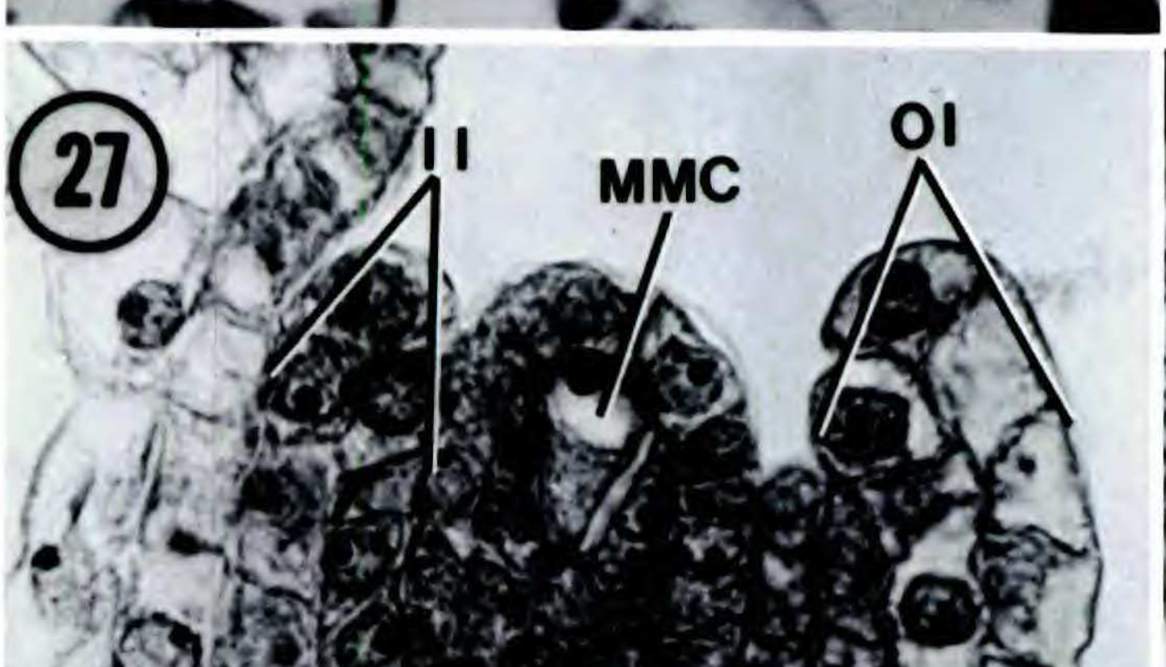
Pellacalyx species

Complete prefertilization embryological data were determined from studies of *P. cristatus* Hemsl. Using the scanty available material of *P. symphiodiscus* Stapf, we confirmed that in anther wall, pollen tetrad, and ovule integument characters, it agreed exactly with *P. cristatus*. Ovules of *P.* cf. *saccardianus* Scort. were examined, and seeds of *P. cristatus* and *P. lobbii* (Hook. f.) Schimp. were studied.

The anther in species of *Pellacalyx* has four microsporangia, the exterior walls of which develop according to the basic-type pattern. The two middle layers degenerate before pollen develops, but the







FIGURES 25–28. Pellacalyx *species.—25.* P. cristatus, *microsporogenesis by predominantly successive meiotic division (M2); some simultaneous divisions are also seen (arrow).* ×485.—*26.* P. cristatus, *microsporogenesis by almost exclusively simultaneous cytokinesis.* ×485.—*27.* P. *cf.* saccardianus, *early ovule. Both integuments initially consist of only two cell layers. Archesporial cell functions directly as megaspore mother cell (MMC); thus, ovule is tenuinucellate.* ×900.—*28. Ovule at anthesis. Except for proliferation of micropylar region, outer integument (OI) remains biseriate, but inner integument (II) has thickened. Nucellus has degenerated; endothelium (ETL) is indicated.* ×315.

epidermis persists to anthesis, although its cells are somewhat collapsed by that time. Secondary wall thickenings are found in the endothecial cells. The tapetum is glandular and composed of binucleate cells. Both successive and simultaneous meiotic cytokineses occur (Figs. 25, 26), producing decussate and tetrahedral pollen tetrads, respectively. Generally, only one type of cytokinesis occurred in any one microsporangium, but incongruent divisions were observed (Fig. 25). Pollen grains are binucleate when dispersed. Anther dehiscence is introrse, occurring by longitudinal slits.

Both integuments of the ovule of *P. cristatus* and *P. saccardianus* are initially two cell layers thick (Fig. 27), but divisions in the inner layer of the inner integument soon form three, then eight to ten cell layers (Fig. 28). The outer integument remains biseriate. Neither integument is vascularized, and both contribute to forming the micropyle. A prominent endothelium is formed during megagametogenesis. The single archesporial cell functions directly as the megaspore mother cell (Fig. 27); thus, the ovule is tenuinucellate. A cell wall is formed after meiosis I, and the megaspore tetrad is usually linear. The chalazal megaspore forms a normal megagametophyte via *Polygonum*-type megagametogenesis. Antipodals are formed but become condensed by the time of anthesis. The polar nuclei remain separate until fertilization. The synergids are pyriform, with prominent chalazal vacuoles.

Discussion

Although this study was limited to one of the two inland tribes of the familiar but poorly understood family Rhizophoraceae, some of the characteristics observed are strongly discordant with embryological dogma and may have significant implications in the study of the control of critical stages of plant development. These features are discussed separately at the end of this section.

Systematic Significance

The data reported here combined with information from other sources (Karsten, 1891; Mauritzon, 1939; Tobe & Raven, 1987a, b, this volume; Juncosa, 1982b, 1984a, b) permit a relatively complete embryological characterization of the family Rhizophoraceae. Reports for African genera of tribe Macarisieae are not yet available, but data from *Cassipourea* and *Sterigmapetalum* (Tobe & Raven, 1987b) are taken as representative of that tribe.

Despite the occurrence of certain derived character states in some genera, the family is remarkably homogeneous embryologically. This contrasts somewhat with the wide range of variation in wood anatomy, mature floral morphology, and chromosome number (Juncosa & Tomlinson, this volume). Allowing for some exceptions, a generalized embryological summary is as follows:

Anthers tetrasporangiate, wall development basic-type (monocotyledonous-type in *Gynotroches*), endothecium with few secondary wall thickenings (more numerous in Macarisieae), middle layers degenerate, tapetum glandular, its cells binucleate; microspore cytokinesis simultaneous (or also successive, in most Gynotrocheae); pollen tetrads tetrahedral or decussate, pollen binucleate. Ovules anatropous, bitegmic, crassinucellate (tenuinucellate in *Gynotroches* and *Pellacalyx*), integuments two cell layers thick at inception (multiseriate in *Carallia* and Rhizophoreae), usually thickening subsequently, endothelium present, micropyle diplostomic, nucellus ephemeral; megagametogenesis chalazal-monosporic (*Polygonum*-type), synergids pyriform with large chalazal vacuoles, antipodals usually degenerate (persistent in *Gynotroches* and *Pellacalyx*). Fertilization porogamous, endosperm initially free-nuclear, embryogenesis variable within species. Mature seeds usually with sclerified exotegmen and abundant nonstarchy endosperm; embryo chlorophyllous, straight, with prominent cotyledons and hypocotyl; radicular vascular cylinder usually medullated; germination epigeal or *Durian*-type.

For most of these characters, the states given above are merely the most common ones among dicotyledons and are regarded as plesiomorphic and therefore not phylogenetically diagnostic. However, the presence of an endothelium is relatively rare in bitegmic ovules (Kapil & Tiwari, 1978). Also, variable orientation of the first and second embryogenetic cell divisions has almost never been reported in families other than Rhizophoraceae, although it may be common in woody plants with large proembryos (Nast, 1941; Souèges et al., 1967). Among temperate herbs, it is apparently common only in Dipsacaceae (Souèges, 1957). Nucellar degeneration, which occurs in all Rhizophoraceae yet studied, is of some diagnostic value. Sclerified exotegmen is not common (Corner, 1976).

The new data reported here support the contention that Rhizophoraceae and Anisophylleaceae are not related (Juncosa, 1982a; Juncosa & Tomlinson, 1987, this volume; Tobe & Raven, 1987a). This latter family differs sharply from the former in all of the apomorphic character states just enumerated (endothelium, degenerate nucellus, exo-

tegmen) as well as in others that are of more general occurrence in dicotyledons. Also, as previously pointed out (Tobe & Raven, 1983), Rhizophoraceae are embryologically out of place in either Myrtales or Cornales, two orders in which the family has often been placed by phylogeneticists (Cronquist, 1968; Thorne, 1976; Takhtajan, 1980). Assignment of the Rhizophoraceae to their own order (Cronquist, 1981; Dahlgren, 1983) merely raises the question of the proper placement of that order. As suggested and discussed by Dahlgren (this volume), the alignment of the Rhizophoraceae with the Celastraceae, Elaeocarpaceae, Erythroxylaceae, and possibly other families is well supported by the embryological data.

These data also have clear implications for intrafamilial systematics. Among the ten genera of Rhizophoraceae so far investigated, only *Gynotroches* and *Pellacalyx* have tenuinucellate ovules and an outer integument that remains two-layered throughout development; the histology of the seed coats in these two genera is similar and differs from that of all other genera in the family (see also Tobe & Raven, this volume). These embryological synapomorphies also correlate with distinctive characteristics of the inflorescence and floral anatomy that are unique to these two genera, such as fasciculate monochasial inflorescences, multiovulate carpels, and idioblastic laticifers (Juncosa & Tomlinson, this volume). Therefore, we conclude that *Gynotroches* and *Pellacalyx* are sister genera, despite the pronounced differences in mature floral morphology that have led some to question even the inclusion of the latter in the family.

As judged by embryological criteria, the systematic position of *Crossostylis* is likely to be intermediate between the Macarisieae and the *Gynotroches/Pellacalyx* clade. The outer integument of the mature ovules of *Crossostylis* is about four cell layers thick, as in *Cassipourea* and *Sterigmapetalum* (Juncosa, 1984a; Tobe & Raven, 1987b). Further, seeds of *Crossostylis* are arillate (Fig. 16; Corner, 1976; Smith, 1981; Tobe & Raven, this volume); seeds of Macarisieae are invariably winged or arillate. This suggests the possibility of merely reassigning *Crossostylis* to the Macarisieae. However, the number of secondary wall thickenings of endothecial cells of *Crossostylis* and other Gynotrocheae is quite low (one to three), whereas five or more such bars are found in *Cassipourea* (Juncosa, pers. obs.). Also, microsporogenesis in *Crossostylis grandiflora* occurs by both simultaneous and successive cytokinesis, a circumstance reported here for *Gynotroches* and *Pellacalyx* but otherwise unknown in the family. Furthermore, there are many important vegetative and floral morphological characters common to *Crossostylis* and other Gynotrocheae that do not occur in Macarisieae (Juncosa & Tomlinson, this volume). Therefore, a reassignment of *Crossostylis* based solely upon the outer integument and aril seems incorrect, despite the importance traditionally ascribed to this character.

Carallia seems also to be phylogenetically intermediate, standing between the *Gynotroches/Pellacalyx* clade and the mangrove tribe (Rhizophoreae). Within the Gynotrocheae, only *Carallia* has a prolonged anther connective and acute sterile tips on the two halves of the anther, characters also found in some Rhizophoreae (Juncosa & Tomlinson, 1987). Nonappendaged seeds and baccate fruits are found throughout the "higher" Gynotrocheae (that is, excepting *Crossostylis*) and Rhizophoreae and thus do not help clarify relationships among these seven genera. Seed coat anatomy of *C. eugenioidea* resembles that of other Gynotrocheae (Tobe & Raven, this volume), but that of *C. borneensis* is vascularized and quite thick, like those of the Rhizophoreae. The seed coat of *C. brachiata* is intermediate between these two in at least some respects (Tobe & Raven, this volume). The seed coat apomorphies of *C. brachiata* (degenerate tegmen) and especially *C. borneensis* may be homoplastic with those of the Rhizophoreae, but it is also possible that the genus *Carallia* is paraphyletic. Embryological data do not resolve this question, but *Carallia* and the Rhizophoreae do share several floral synapomorphies (Juncosa & Tomlinson, this volume and unpubl. obs.), and only one putative autapomorphy distinguishes the genus *Carallia* (stalked extrastipular glands). This character is known to occur only in *C. longipes* (Ding Hou, 1960) and *C. borneensis* (Juncosa & Tomlinson, this volume), but other species have yet to be examined critically; if these glands prove not to be ubiquitous in the genus, its monophyly will be questionable. With the possible exception of the vascularized integument of *C. borneensis*, we observed no embryological peculiarities in *Carallia* that could be construed as any kind of preadaptation to vivipary, which therefore appears to have arisen entirely within the mangrove tribe.

DEVELOPMENTAL IMPLICATIONS

It is generally believed that nearly all fundamental embryological characters are invariant for a given species (Davis, 1966). For example, the extensive literature on angiosperm embryogenesis

is based upon this supposition (Schnarf, 1929; Johansen, 1950). However, variability in cell division pattern has been reported in two genera with relatively large embryos (*Juglans*: Nast, 1941; *Laurus*: Souèges et al., 1967) and is characteristic of all Rhizophoraceae yet studied (Juncosa, 1982b, 1984a, b). Interestingly, among Rhizophoraceae, the genus that most consistently exhibits the familiar onagrad-type pattern (as traditionally described) is *Gynotroches*, which has quite small embryos in comparison with those of other genera. This suggests a correlation between small embryo size at cotyledonary initiation and consistency in orientation of cell divisions that has not previously been recognized. Unfortunately, most of the embryogenetic literature pertains to temperate, herbaceous species with small seeds and embryos, so it is not clear from this limited example whether this correlation is generally true or not.

Another embryological characteristic that may have developmental implications is variability in meiotic cytokinesis in the anthers of all Gynotrocheae except *Carallia*. This has not previously been reported in any angiosperm, although the variation in pollen tetrad configuration that has occasionally been noted (Davis, 1966) suggests that variable cytokinesis may occur widely. However, it is not merely the occurrence of this variation in Gynotrocheae, but especially the pattern of variation that is developmentally significant: nearly all meioses in a single microsporangium occur by only one cytokinetic pattern, yet adjacent sporangia in a single stamen may exhibit different patterns (Figs. 12, 13, 18, 19, 25, 26). This suggests strongly that the pattern of cytokinesis is controlled by tapetal secretions, not by the individual microspore mother cells. Further investigation of this system is certain to yield significant new insights into the process of meiosis in angiosperms.

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